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THE EARLY DEVELOPMENT OF THE PIGEON'S EGG, WITH ESPECIAL REFERENCE TO THE SUPERNUMERARY SPERM NUCLEI, THE PERIBLAST AND THE GERM WALL.

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A Preliminary Paper.

In the *American Journal of Anatomy*, September, 1904, there appeared a paper by Dr. E. H. Harper on "The Fertilization and Early Development of the Pigeon's Egg." Dr. Harper found that the egg is polyspermic; that one sperm nucleus unites with the egg nucleus; and that the supernumerary sperm nuclei migrate to the periphery of the germinal area and there set up an accessory cleavage. He followed through the development to the sixteen-cell stage, or about eight hours after fertilization, and although he gives two figures of sections of an egg fifteen hours after fertilization, the intervening stages were not filled in.

At the zoölogical laboratory of the University of Chicago, in January, 1905, I took up the study of the pigeon's egg, hoping to continue from the sixteen-cell stage. But in order to appreciate the material, it was necessary to go back into earlier stages. I have obtained an egg for every hour of development from the formation of polar bodies to the time of laying—a period of about forty-one hours. For some of the more critical stages before laying I have more abundant material, and also have a good many laid eggs.

The purpose of this preliminary paper is to announce some of the more important steps in the early development, but without

presenting the abundant proof which the material affords for my conclusions.

Through the kindness of Prof. C. O. Whitman and other members of the Department of Zoölogy, I have been the recipient of a university fellowship which has enabled me to pursue this study. Dr. F. R. Lillie, at whose suggestion I undertook the research, has followed the work carefully, and I thank him for his interest and kindness. I am also indebted to Mr. W. L. Tower for his help in photography. The living egg is a difficult subject, and it was only after a great many efforts that I secured any photographs. Twelve cleavage stages have been photographed, although only three are presented in this paper.

Methods.

Following the method of workers who have preceded me, the blastoderm has been killed and hardened on the yolk, and the orientation marked with a bristle: Immediately after a window has been made through the shell, a bristle is inserted in the side of the yolk toward the blunt pole of the shell. Later (usually when the egg is in 70 per cent. alcohol) a five-sided piece, including the blastoderm, is cut out from the yolk. One side of the five-sided area is perpendicular to the chalazal axis, and is toward the large pole of the egg. Two sides are parallel to each other and to the chalazal axis, and the last two sides meet in a

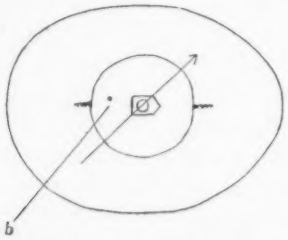

Fig. 1. Diagram to show the method of marking the orientation. The arrow indicates the direction of the axis of the future embryo. *b*, bristle.

sharp angle pointed toward the small pole of the egg. Fig. 1 makes this orientation clear, the anterior side of the blastoderm being toward the point of the arrow. This five-sided block is

easily seen in the paraffin cake for orientation in cutting. Kleinenberg's picro-sulphuric acid (strong solution) plus 10 per cent. acetic has been the most successful for killing and fixing, although other solutions have been used.

Fertilization.

Dr. Harper (3) found that the egg is fertilized in the evening, at the time it leaves the ovarian capsule and enters the oviduct and he makes this statement, "In all cases observed, this has taken place between seven and nine o'clock." I shall, therefore, refer to eight o'clock in the evening as the hour of fertilization, although the exact time for any particular oviducal egg is not known.

To present the history of the early development, I shall describe several critical stages as follows:

1. Position of the supernumerary sperms at the close of maturation.
2. The 8-cell stage.
3. The 16-cell stage.
4. The last stage of the multiplication of the sperm nuclei.
5. The disappearance of the sperm nuclei.
6. The periblast.
7. The growth of the blastodisc at the expense of the periblast.
8. The germ wall.

1. *Position of the Supernumerary Sperm Nuclei at the Close of Maturation.*— In an egg taken from the oviduct at 11:30 P. M., or about three and one half hours after fertilization, the first cleavage plane had not formed. In this egg, the supernumerary nuclei had migrated into the periblast at the periphery of the germinal area, and they occupied a circle which in later stages is indicated superficially by accessory cleavage. Some of these nuclei were in mitotic division.

2. *The 8-cell Stage.* — Abundant material has been obtained in stages of two and four cells, but for brevity, a description of those stages is omitted from this paper.

At 4:45 A. M., eight and three fourths hours after fertilization, an egg of eight (or perhaps nine) cells was taken from the shell gland. Its surface view is shown in Fig. 2, and a transverse

section in Fig. 3. The dotted circle, Fig. 2, represents the distance to which the sperm nuclei may migrate peripherally (it is also the peripheral limit of the periblast nuclei, to be explained later), but the accessory cleavage, with a few exceptions, is con-

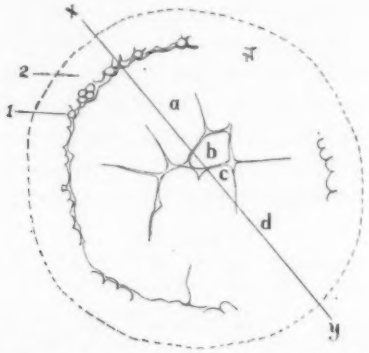

FIG. 2. Sketch of surface view of a pigeon egg about eight and three fourths hours after fertilization, 4:45 A. M. *a*, *b*, *c*, *d*, cells of primary cleavage which are shown in section in Fig. 3. *xy*, the plane of the section in Fig. 3. 1. Accessory cleavage. 2. Periblast.

fined to the zone just outside the blastomeres of the primary area. A migrating sperm nucleus is shown at the extreme right of Fig. 3. Another sperm-nucleus has migrated under the large blastomere *a*. It is in the central periblast, which will be explained later. A study of the whole series of sections showed a number of nuclei in this position, forming a submarginal circle. They migrate later as far centrally as the margins of the nucleus of Pander, but were never found under the very center of the blastoderm.

Fig. 3 is of a section taken through the plane *xy* of Fig. 2.

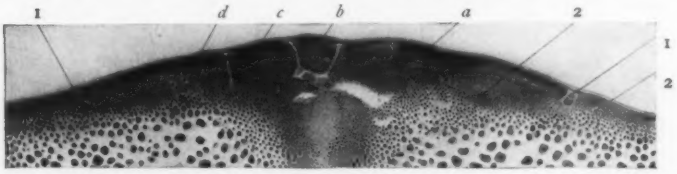

FIG. 3. Transverse section of the pigeon egg whose surface view is shown in Fig. 2. *a*, *b*, *c*, *d*, cells of primary cleavage. 1. Accessory cleavage. 2. Migrating sperm nuclei.

The cells of this section will be recognized as the cells with the corresponding letters in Fig. 2. The small cell *b* is cut off from the underlying yolk in this section, but it is so only at its central end. In sections anterior to this, the cell *b* is continuous with the yolk. The split made by this horizontal cleavage marks the position of the *future segmentation cavity*. This horizontal plane may not be permanently established at this stage. It seems to come and go during the next few hours of development. But *its position indicates the depth of the center of the blastoderm in cleavage stages.*

A comparison of the several stages here represented (Figs. 3, 5, 6 and 9) by actual measurement of the drawings will show that there is but the slightest variation in the depth of the germinal disc at the center.

In contrast with this, is the account of the hen's egg by Kölliker (6). He describes the blastodisc as increasing in depth as cleavage progresses. In his Fig. 19 (which represents a vertical section through a hen's egg of about twenty cells) two central "*Furchungskugeln*" and two marginal "*Segmenten*" are shown; *i. e.*, there are four cells in the section forming a single layer. Between this layer of cells and the white yolk is the unsegmented "*Bildungsdotter.*" None of these products of cleavage is completely cut off from the "*Bildungsdotter.*" They form a layer .14 mm. in depth in the center. A section through a later stage in the development of the hen's egg is shown in Kölliker's Fig. 22 where, "*die Dicke der durchfurchten Stelle in der Mitte des Keimes gerade noch einmal so dick war, als in dem früher beschreibenen Fälle* (*Fig. 19*) *nämlich 0.28–0.30 mm.*" . . . "Somit greift die Durchfurchung, indem sie weiterschreitet, in der Mitte der Keimschicht immer mehr in die Tiefe, wie schon Oellacher dies vermuthet hat, und erreicht am Ende nahezu die Grenze der Lage die in der Fig. 19 mit *bd* als ungefurchten Bildungsdotter bezeichnet ist."

Kölliker suggests that the adding of cells from below may be by a process similar to the adding of cells to the central part from the marginal segments,—*i. e.*, the nucleus of a marginal segment divides and the central end of the segment containing one of the daughter nuclei is cut off and becomes a "Furchungs-

kugel." The other daughter nucleus passes into the marginal segment, and so on until finally the part of the marginal segment left over, changes over into a "Furchungskugel." And so, according to Kölliker, the first appearing *Furchungskugeln* are never completely cut off from the unsegmented *Bildungsdotter* below, but nuclei, sisters to those in the first layer of cells, pass down into the *Bildungsdotter*. Here nuclear division takes place, and cells are organized around the upper daughter nuclei, thus forming the second layer of cells in the center of the blastodisc, while the lower daughter nuclei are left deeper in the "Bildungsdotter." And thus cleavage proceeds downward until finally the last remaining nucleated portions of the "Bildungsdotter" change over into "Furchungskugeln."

In the pigeon's egg, on the contrary, I do not find any such deepening of the center of the blastodisc. The change from one layer to several layers of cells is by a process *exactly like that of the teleost egg*. See Agassiz and Whitman (1) Fig. 2, and Wilson (8) Figs. 16, 17, 18 and 19. *The blastodisc of the pigeon's egg becomes stratified by horizontal cleavage planes arising above the first horizontal cleavage; i. e., above the level of the plane which limits the cell b below* (Fig. 3). *Nuclei are never found in the central part below the level of the horizontal cleavage under the cell b.*

Extending deep into the white yolk is a cone of slightly granular protoplasm. It varies in extent in different stages as will be seen by comparing Figs. 3, 5, 6 and 9. A more central section than Fig. 5 shows this cone extending deeper. In some stages it is better described as being funnel-shaped, with the slender tube of the funnel going deep into the yolk, and the mouth opening on the the lower side of the blastodisc. In some of the sections of the egg represented in Fig. 9 it is found at twice the depth of the figure. If the sections were cut exactly perpendicular to the surface, the funnel would appear continuous from its broad end at the blastodisc to the deepest limit included in the section. A similar structure has been figured by Eycleshymer (2) for the egg of *Lepidosteus osseus*, Figs. 32, 34 and others. He calls it, "the peculiar conical prolongation of the periblast."

3. *The 16-cell Stage.* — Fig. 4 is a photograph of a pigeon egg of sixteen cells. Although of later development, it was obtained an hour earlier than the egg shown in Fig. 2, — *i. e.*, it was taken from the oviduct at 3:45 A. M., seven hours and forty-five minutes after the time from which fertilization is reckoned. The arrow indicates the direction of the axis of the embryo, and the anterior side of the blastoderm is in the direction of the point of the arrow.

Three principal regions in the blastoderm of the bird's egg are to be recognized in surface view at this stage: (1) the *central area*, (2) the *marginal cells*, (3) the *periblast*. In this egg (Fig. 4), the central area is occupied by six cells [the Furchungskugeln of Kölliker (6)] and there are ten marginal cells (Kölliker's Segmenten). The periblast is the zone outside the marginal cells. At the inner margin of this zone is the accessory cleavage caused by the supernumerary sperm nuclei. This is on all sides, but at a few places where there are no sperm nuclei, the large marginal cells are open peripherally.

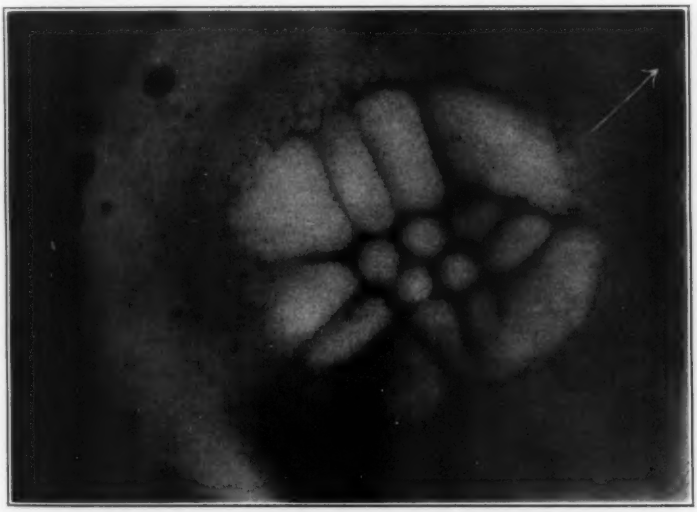

FIG. 4. Photograph of a pigeon's egg 7¾ hours after fertilization. 3.45 A. M. The anterior side of the blastoderm is toward the point of the arrow.

As cleavage proceeds cells are cut off centrally from the marginal cells, and added to the central area, and thus the latter grows at the expense of the former (compare Fig. 7). Radial cleavage planes divide the marginal cells and increase their number, while the central cells are constantly becoming smaller by division. Finally, the marginal cells are all used up, and we recognize only two regions in the blastoderm, (1) the central area, and (2) the periblast. In early stages, all of the cells are continuous with the yolk, but as development proceeds, the central cells become complete below and separate from the yolk, and only the marginal cells are open below. Thus the marginal cells constitute a "zone of junction" (see Agassiz and Whitman (1), Figs. 2, 3, 4 and 5) between the segmented and unsegmented parts of the egg. All of the photographs presented in this paper show a very symmetrical form of cleavage, and while I have found a good many instances of asymmetrical cleavage, I cannot agree with Kölliker (6) that "Die Furchung geht immer asymmetrisch vor sich, so dass ohne Ausnahme die eine Hälfte der Keimscheibe in der Zerklüftung der anderen voran ist."

4. *The Last Stage of the Multiplication of the Sperm Nuclei.* — In an egg obtained at 6:30 A. M., ten and a half hours after fertilization, the sperm nuclei were very numerous. There is no record of the exact number of cells of primary cleavage showing

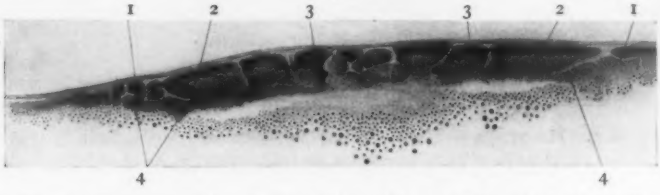

FIG. 5. Transverse section of a pigeon's egg at the end of the period of multiplication of the sperm nuclei. Egg taken 6.30 A. M., about 10 hours after fertilization and 31 hours before laying. Note that all cells are still continuous with the yolk. 1. Accessory cleavage around the sperm nuclei. 2. Marginal cells sharply separated from the sperm nuclei. 3. Central cells. 4. Sperm nuclei.

on the surface of this egg, but there were a few more than thirty-two. The accessory cleavage was very abundant and more than one cell in depth. A transverse section through about the cen-

ter of this blastoderm is shown in Fig. 5. The accessory cleavage is confined to the region immediately outside of the large marginal cells of the blastoderm, but the sperm nuclei have migrated peripherally into the unsegmented part. These nuclei were more abundant than this drawing suggests; for on the right hand side of the section there were four more nuclei in superficial positions in the unsegmented part beyond the limits of the figure. The sperm nuclei were just as abundant in every other section of this egg. But these nuclei have migrated not only peripherally; they are also under the large marginal blastomeres. The latter, however, are definitely separated from the sperm nuclei by cleavage planes whose significance will be better appreciated in contrast with a stage after the disappearance of the sperm nuclei as shown in Figs. 6 and 9.

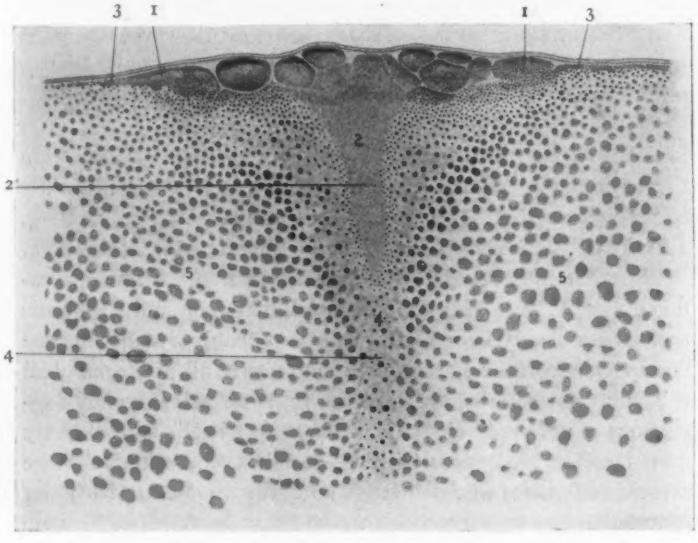

FIG. 6. Longitudinal section of pigeon's egg at the time of disappearance of the sperm nuclei; on the left (anterior), the marginal cell has become open, *i. e.*, continuous with the marginal periblast. On the right the marginal cell is still slightly separated from the periblast at the surface. Surface view of the egg showed traces of accessory cleavage; note continuity of the central cells with central periblast. 1. Marginal cells. 2. Cone of protoplasm. 3. Marginal periblast. 4. Neck of latebra (white yolk). 5. Yellow yolk. Egg taken 7 A. M., about eleven hours from fertilization (estimated).

5. *The Disappearance of the Sperm Nuclei.* — Fig. 6 represents a longitudinal section through about the center of the blastoderm of an egg taken from the oviduct at 7:00 A. M., or eleven hours after the approximate time of fertilization. There were in this egg a few remaining sperm nuclei, and where they occurred, they were separated from the marginal cells by cleavage planes similar to those on the right of Fig. 5. In sections where the sperm nuclei did not appear, the marginal cells were open to the *periblast*, as on the left of Fig. 6. On the right of this figure (which is the posterior side of the blastoderm) the marginal cell is partly closed in, and in a few sections beyond this, it was entirely closed, being separated from a cell of accessory cleavage. In surface view, also, the marginal cells were open peripherally except where the accessory cleavage occurred.

In another egg taken from the bird at 7:00 A. M. (eleven hours from fertilization) *every marginal cell as seen in surface view was open peripherally, and in sections, the margin was like that at the left of Fig. 6. Not one nucleus was found outside the cells of primary cleavage.*

Other eggs of about this period show accessory cleavage on the wane and conditions in sections like those in Fig. 6. In the egg represented in Fig. 5, the sperm nuclei were fragmenting. They disappear between ten and twelve hours after fertilization.

Fig. 7 is a photograph of an egg eleven hours from fertilization (7:10 A. M.). Here, the central, marginal and periblastic regions are clearly expressed. This is probably a stage after the disappearance of the sperm nuclei, and nearly all of the marginal cells are open peripherally. At the posterior side there are suggestions of accessory cleavage. These small cells are probably mere bud-like projections from the periblast, and not due to the presence of sperm nuclei. It is impossible to decide this point from surface view, but sections would show the relations between the marginal cells and the periblast, and therefore demonstrate whether the nuclei of these small cells were derived from supernumerary sperms or from the cleavage nucleus.

6. *The Periblast.*— Previous paragraphs have anticipated the discussion in this. Any mention of the periblast refers the student of vertebrate embryology to the work of Agassiz and Whitman

(1) on *Ctenolabrus* where the origin of the periblast was first accurately described. Only the twelve marginal cells of the sixteen-cell stage of the teleost egg rest upon the yolk. The contact with the yolk is at the inferior outer angle of the cells, and this region "may be designated as the zone of junction" (Agassiz and Whitman) between the blastodisc and the

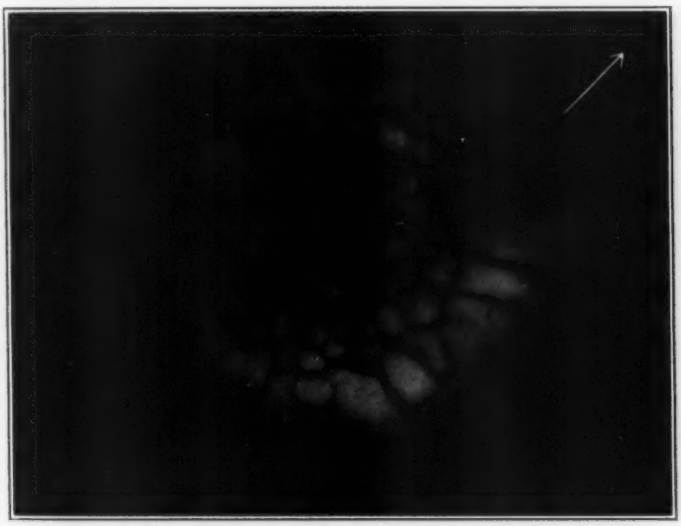

FIG. 7. Photograph of pigeon's egg 11 hours after fertilization, 7.10 A. M. The point of the arrow indicates the anterior side.

periblast. The marginal cells of the teleost are open peripherally. Now, there is to be recognized in the bird's egg a periblast exactly comparable at this stage (eleven or twelve hours after fertilization) with the periblast of the fish egg. We may think of a *potential periblast* in the *unsegmented pigeon's* egg. Into this the sperm nuclei migrate.

After these nuclei disappear the marginal cells of the blastodisc open peripherally to the periblast and are directly continuous beneath with the yolk. The nuclei of the marginal cells divide, and some of the daughter nuclei migrate into the unsegmented region, and thus the periblast "becomes cellular," to use the ex-

pression of Agassiz and Whitman (1). The periblast nuclei migrate peripherally and also into subgerminal positions, and thus we may speak of a *marginal* and *central* periblast. But the nuclei of the central periblast have not been found in the nucleus of Pander.

In *Lepidosteus osseus*, Eycleshymer (2) found the nuclei most numerous at the center, "undergoing rapid division and contributing one derivative to the cell cap.

Fig. 8 is a photograph of an egg obtained at 9:30 A. M., or thirteen and a half hours after fertilization. The marginal cells

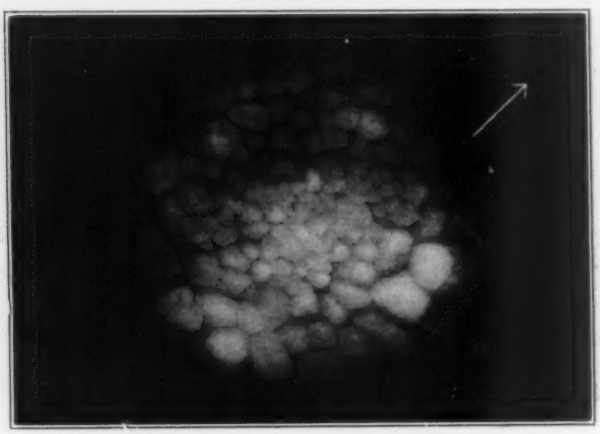

FIG. 8. Photograph of pigeon's egg 13½ hours after fertilization, 9.30 A. M. Anterior side of blastoderm toward point of arrow.

are now limited peripherally, but are open below as is suggested in Fig. 9, a transverse section through another egg of about the same age. The periblast in such an egg as Fig. 8 is demonstrated only in sections. ' It does not appear in surface view.

7. *The Growth of the Blastodisc at the Expense of the Periblast.* — In the teleost egg, after the conclusion of cleavage, the periblast remains distinct from the blastodisc, but in the pigeon's egg, the periblast continues to add cells to the segmented region. I have studied this point carefully up to several hours after laying, but have not completed my study on later stages of incubation. To illustrate this, there is a series of drawings, Fig. 9 to Fig. 15.

The transverse section represented in Fig. 9 is through about the center of the blastoderm of an egg taken from the bird at 10:30 A. M., or about fourteen and a half hours after fertilization. The center of the blastodisc has become three cells deep, and is separated from the yolk by a sharp line bounding the latter. But the marginal cells are continuous with the yolk, and

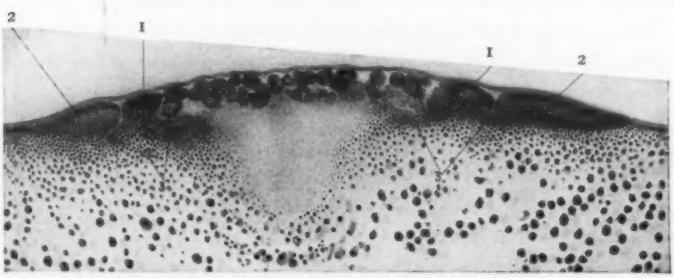

FIG. 9. Transverse section through the center of the blastoderm of a pigeon's egg taken at 10:30 A. M., 14½ hours after fertilization. 1. Marginal cell. 2. Marginal periblast. 3. Nuclei in the central periblast, derived from the nucleus of the marginal cell.

protrusions from the central periblast extend into the segmentation cavity. Nuclei are often found in these protrusions, which suggest that cells are being added to the segmented part. This egg is still in the cleavage stage, being twenty to twenty-two hours before gastrulation [considering the time of gastrulation five to seven hours before laying as determined by Mr. Patterson (7)], and may therefore be considered not unlike the teleost egg. But in following through the successive later stages, similar relations are found between periblast and blastodisc and there is no time when they are distinct.

Fig. 10 shows in mere outline the conditions at the margin of

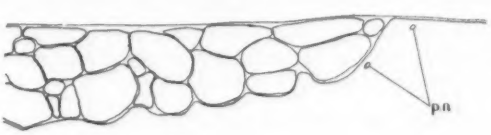

FIG. 10. Margin of a transverse section of a pigeon's egg about twenty and a half hours after fertilization, 4:25 P. M. *p.n*, periblast nuclei.

the blastoderm at 4:25 P. M., or twenty and a half hours after fertilization. Some of these cells at least have been derived by division of such a marginal blastomere as shown in Fig. 9. Others may have been derived from the periblast, with nuclei sisters to those yet remaining in the unsegmented part.

Fig. 11 is the posterior end of a longitudinal section through an egg perhaps twenty-five hours after fertilization (8:50 P. M.) Four nuclear nests and two single nuclei are found in the periblast. Beyond the limits of the drawing, are four other nuclei

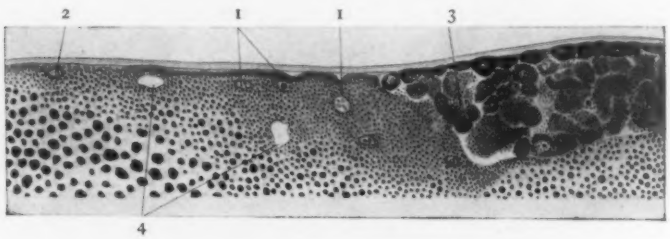

FIG. 11. Posterior side of a longitudinal section of a pigeon's egg about twenty-five hours after fertilization, 8:50 P. M. 1. Nests of periblast nuclei. 2. Periblast nucleus. 3. Syncytial mass derived from the periblast, organizing into cells which will be added to the blastodisc. 4. Vacuoles.

two of them are in line with the most extreme nucleus to the left and two are a little deeper. Large masses, as shown at 3, Fig. 11, are organized out of the periblast and subsequently they divide into smaller cells. Indentations just to the left of the segmented part here suggest future cleavage which would add superficial cells. (Compare Fig. 14.) This figure (Fig. 11) resembles Harper's (3) Fig. 36 which is a section of an egg fifteen hours after fertilization. Harper considers that the "free nuclei" are sperm nuclei but there was a gap in his material just at the period when the sperm nuclei disappear and the periblast is organized. The nuclei of his Fig. 36 are doubtless periblast nuclei.

Fig. 12 shows a marginal part of a horizontal section through an egg of the same age as Fig. 11 (twenty-five hours after fertilization, 8:50 P. M.). Here are "free nuclei" or periblast nu-

clei and such relations between the segmented and unsegmented part as to suggest contribution of cells from the periblast. Of course, such segregation of cytoplasm and granules around nuclei as is indicated at 2 and 3 may not be permanent. The nucleus for the cell at 3 is in the next section. Amitotic division is suggested by some of the nuclei. Compare the position of

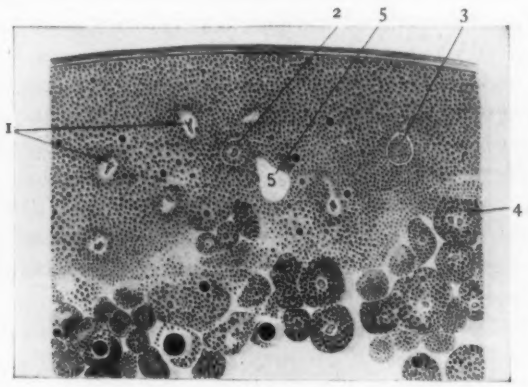

FIG. 12. Marginal part of a horizontal section through a pigeon's egg about twenty-five hours after fertilization, 8:50 P. M. 1. Periblast nuclei. 2 and 3. Cells being organized out of the syncytium. The nucleus for 3 is in the next section. 4. A cell contributed from the periblast. 5. Vacuole.

nuclei in a syncytial zone outside the segmented blastodisc with the condition shown in Wilson's "Embryology of the Sea Bass" (8), Figs. 23 and 24.

The following outline drawings, Figs. 13, 14, and 15, need

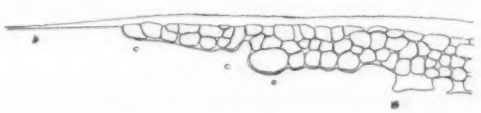

FIG. 13. Margin of a transverse section of a pigeon's egg, about 26 hours after fertilization. Notice cells being added to the segmented part from the periblast. The periblast nuclei were not all in this section, but were found in four successive sections. Two nuclear nests are shown.

little explanation. They are of transverse sections of eggs about 26, 28 and 32 hours respectively after fertilization. They sug-

gest the spreading of the blastoderm over the unsegmented part by cells organized around the superficial periblast nuclei. The blastoderm instead of having an almost perpendicular margin as in Fig. 10, comes to lie over the periblast. These figures show other additions of cells besides those at the extreme margin.

8. *The Germ Wall.* — The term *Keimwall* was first used by His in 1866. In his description of the germ wall of the hen's egg, His (5) says that in the first hours of incubation the white yolk on which the border of the germ area rests is grown through with cells of the germ, and it forms a peculiar structure with protoplasmic frame work enclosing white yolk spheres. To this structure, His gave the name "*Keimwallgewebe*" or *organisirten Keimwall.*

His (5) also says that he was able to follow "wie tiefliegende Zellen des Keimes vermöge ihre sehr ausgesprochenen amöboiden Beweglichkeit die ihnen benachbarten Dotterkörner und Dotterkugeln in sich aufnehmen."

In another paper His (4) says, "Während der ersten Zeit der Bebrütung entsendet die untere Schicht jenes Randtheils Fortsätze zwischen die Elemente des Keimwalles, so dass diese grossentheiles in ein Gerüst archiblastischen Protoplasmas eingeschlossen werden."

This conception of His is based upon a study of hen's eggs during the first few hours of incubation. He makes no reference to the germ wall in the unlaid egg. A study of the pigeon's

FIG. 14. Margin of transverse section of a pigeon's egg about 28 hours after fertilization. The periblast nuclei, except the most peripheral one, were all in this section.

egg in close stages of development before laying gives quite a different conception of the germwall, — particularly as to the origin of the nuclei. They are *periblast nuclei,* and are not derived from the "tiefliegende Zellen des Keimes." Such nuclei are shown in Fig. 16 which represents the margin of the blastoderm in transverse section of a pigeon egg six hours before laying. It is, of course, a younger stage of the germ wall than is

represented in any of the figures by His (5). Moreover, none of his figures through the germ wall of the hen's egg show the extreme margin of the blastoderm. The nuclei in the unsegmented part in Fig. 16 are periblast nuclei, and their history can be traced back through each preceding stage of development to a period about eleven or twelve hours after fertilization when nuclei from the marginal cells pass into the periblast. Indeed, such a history of the nuclei may be retraced through the figures of this paper — Figs. 16, 15, 14, 13, 11, 10, 9, 6.

As development proceeds from such a stage as is represented in Fig. 9, the zone of junction established by the marginal cells between the blastodisc and the periblast travels outward and the blastodisc increases in diameter as cells are added to its margin

FIG. 15. Margin of a transverse section of pigeon's egg about 32 hours after fertilization. The periblast nuclei were all in this section. One nuclear nest is shown. Notice additions from the periblast to the segmented part.

from the periblast. Cells are organized around the superficial periblast nuclei and sisters to these nuclei are left deeper in the unsegmented periblast. Thus the extreme margin of the blastodisc is thin, Figs. 13, 14 and 15. But later, the deeper sister-nuclei are enclosed in cells and so the blastodisc thickens up under that part which had been only one layer of cells in depth. But, meantime, the *thin margin* has advanced over the yolk, by addition of cells from the periblast. However, there comes a time a few hours before laying (Fig. 16) when the *margin* thickens up. This, I think, is the condition described by His (5), "Am umbebruteten Keim sind die Zellen der unteren Keimschicht von denen der oberen nicht allzusehr verschieden. In dem Randtheil eines unbebrüteten Huhnerkeimes gehen obere und untere Keimschicht in einander über und sie sind nahezu gleich dick. Die untere, lockerer gefügt als die obere, ist eher etwas schwächer. . . . Dotterkörner finden sich auch in Zellen der obern Schicht, obwohl nicht sehr reichlich."

In other literature it is said that the *lower germ layer* forms a

compact mass with the *germ wall*, which, like a thickened border, rests upon the yolk. This thickened border also receives the name *Randwulst* and *bourrelet blastodermique*.

I would describe the margin of the blastoderm (Fig. 16) not as a region where the *upper and under germ layers go over into each other, but as a syncytial region out of which two layers of cells differentiate centrally*.

Through each period of development leading up to this stage (as is shown by the series of figures in this paper), the periblast nuclei keep ahead of the advancing margin of the blastodisc. They multiply and a part of them are used up in the cells that are continually being added to the margin. Such a nucleus in *advance* of the margin of the blastodisc is shown at the left of

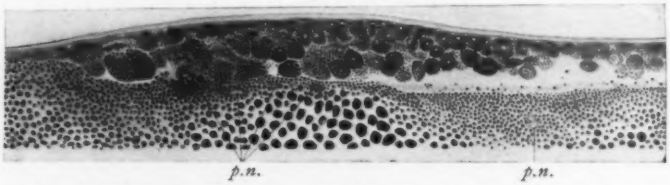

FIG. 16. The germ wall in transverse section through the center of the blastoderm of a pigeon's egg, 8:10 A. M., 36 hours after fertilization and 6 hours before laying. *p.n*, periblast nuclei. They were not all found in this section, but were reconstructed from five successive sections. There are six other periblast nuclei in this half of the section, but in positions central to the limits of the figure. The right hand side of the figure is toward the center of the blastoderm.

Fig. 16. It is in the periblast. It is not enclosed in a cell — *i. e.*, it is not separated by a cleavage plane from the periblast which extends further peripherally — other periblast nuclei are *below* the margin. The thickened-up character of the margin is due to the *upward differentiation* of cells from the periblast. It is not a region where the blastodisc is deepened by the opening of the lower layer of cells to send protoplasmic processes into the white yolk. The cells of this region, which are open below, are so *because they have not yet, in the process of their differentiation out of the periblast, become closed*. Large nucleated masses differentiate *upward* from the periblast. These masses become multinucleate, and finally divide up into several cells. As this region becomes older, that is, as it is left behind while the margin of the

blastoderm advances, the cells individualize and separate from each other. The whole thickened margin is a *syncytium*. It is an *embryonic region* whose depth is measured from the *vitelline membrane* to the lowest limit of the periblast. There is not an ectoderm extending over this region to the extreme margin of the blastoderm. The cells next to the vitelline membrane, are, of course, flattened against it, but their lower border does not give the character of an epithelial layer. Only on the side of this syncytium toward the center of the blastoderm do the cells next the vitelline membrane form a true epithelium. From this thick, syncytial border region cells *individualize centrally*, and form two layers — (1) an upper layer, the ectoderm, and (2) a lower layer of loosely arranged cells.

It is not difficult to explain the presence of yolk-granules in the cells of the upper layer because these cells, like all others in this region are derived from the periblast in which the yolk-granules are abundant.

The section whose margin is shown in Fig. 16 presents two layers of cells in the central part, which roof over a large cavity filled with fluid. The marginal part of the cavity is shown in the figure. Periblast nuclei are everywhere under the blastodisc except in the nucleus of Pander. From them are derived the nuclei which are, in later stages of incubation, in the germ wall of the area opaca (His) and in the Randwulst. I shall not at present attempt to discuss these later stages, but in a more complete paper, I shall present further evidence in support of this conception of the germ wall and shall describe it in other than transverse sections, and shall also describe the formation of the vascular layer.

Summary.

1. The supernumerary sperm nuclei migrate into the potential periblast and disappear between ten and twelve hours after fertilization.

2. The position of the cleavage cavity is indicated by the first horizontal cleavage.

3. After the disappearance of the sperm nuclei, the marginal cells open peripherally and the periblast becomes organized with nuclei derived from the cleavage nucleus.

4. Cells are added to the blastodisc from the marginal and central periblast.

5. The "free nuclei" under the blastodisc are periblast nuclei. They are the nuclei of the germ wall, including the *Randwulst.*

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ON GASTRULATION AND THE ORIGIN OF THE PRIMITIVE STREAK IN THE PIGEON'S EGG. — PRELIMINARY NOTICE.

J. THOS. PATTERSON.

The results of the experimental studies of Assheton ('96), Miss Peebles ('98), and Kopsch ('02) on the primitive streak of the chick demonstrate beyond any reasonable doubt that the material of that structure enters into the formation of the embryo —a view long held by many embryologists. In the light of these experiments the opposite view of Balfour and his followers is no longer tenable. The results obtained by these three workers have, in the main, solved the problem of the fate of the primitive streak, but they have not answered the question of its origin. The present work was undertaken with the hope of throwing light upon the latter question. It was soon found that its solution depended upon a morphological and experimental study of stages occurring before the time of laying. The morphological results mainly will be considered in this paper.

Material and Methods.

It is doubtful if a more desirable material could be found for the purposes of this investigation than that furnished by the pigeon's egg. The regularity of the laying habits of the common pigeon makes it possible to secure eggs at approximately any stage of development. Breeders have long known that this bird ordinarily lays two eggs at a sitting, the first usually between four and six P. M., and the second between one and two P. M., on the second day following. According to Harper ('04) this latter egg is fertilized at about eight P. M., just before it enters the oviduct, and hence it is forty-one hours in traveling down this passage. Thus, it will be seen that the investigator can secure this second egg at approximately any stage of its early development, for he needs but kill the bird at the proper hour and remove the egg from the oviduct in order to obtain a desired stage. Eggs removed in this manner, even as early as twenty hours before lay-

ing, can be used for experimentation; for by this time the shell is firm enough to permit handling without injury to the blastoderm.

In dealing with this material it was necessary to use special technique both for fixing and orientation. The picro-sulphuric-acetic mixtures have been found to be vastly superior to all other reagents. Of these mixtures the most successful is 92 parts of Kleinenberg's strong picro-sulphuric plus 8 parts of glacial acetic. The whole yolk is immersed in this fluid for one hour and is then treated with 70 per cent. alcohol for several hours, after which it is placed in 80 per cent. At this point it is found advisable to cut out a properly oriented wedge-shaped block of yolk containing the blastoderm, with vitelline membrane still attached. After completely washing out the picric acid, this block is carried through the higher alcohols, cleared in cedar oil, and embedded and sectioned in the usual way.

For stages prior to the appearance of the primitive streak, such a treatment necessitates a careful orientation of the blastoderm before using the fixing fluid. Already a method for orienting the chick blastoderm has been worked out. Thus a number of investigators have shown that if a hen's egg be held in front of the observer so that the blunt end is to his left and the pointed end to his right, the posterior margin of the blastoderm will be towards and the anterior away from him, and hence, when the embryo appears, its head will be directed away from the observer, with its long axis meeting the chalazal axis at right angles. If a pigeon's egg be held in a similar position a different condition is found. The posterior margin of the blastoderm, instead of being directly in front of the observer, is forty-five degrees to his left, and when the embryo arises, its long axis meets the short axis of the egg at an angle of forty-five degrees (see Fig. 1).

For some purposes iron hæmatoxylin has been of great value as a stain, but for general use a modification of Delafield's hæmatoxylin is unsurpassed, especially for demonstrating the presence of cell walls.

Gastrulation.

I stated above that it was necessary to investigate the period of development that occurs before laying. A study of these early stages naturally involves the question of the origin of the two

primary germ layers. Concerning the manner in which these two layers arise there has been a wide difference of opinion among embryologists, although a great deal of attention has been paid to this question. The unsatisfactory solution of this problem is due to the fact that most of the conclusions are based on incom-

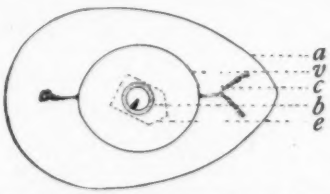

FIG. 1. Scheme for orienting the blastoderm of the pigeon's egg in cutting sections. *a*, shell; *b*, blastoderm at the first appearance of the primitive streak; *c*, chalaza; *v*, vitelline membrane; *e*, wedge-shaped block of yolk containing the blastoderm which is cut out and embedded for sections.

plete evidence. So far as I am aware, not a single observer has had a complete series of normal stages of any one type from which to draw his conclusions. The divergent views as to the origin of the entoderm, however, can be grouped into three classes. (1) A number of the older workers have maintained that it arises by a process of delamination, that is, the upper cells of the segmented disc arrange themselves into a continuous layer, constituting the primary ectoderm, while the deeper cells of the disc form the primary entoderm. This view is not in accord with what is known to occur in many other forms. (2) Others have maintained that the entoderm arises by an ingrowth of cells into the segmentation cavity from a part or all of the inner edge of the germ-wall. The most recent contribution supporting this view is by Nowack ('02) who states that the bulk of the entoderm is formed out of a mass of cells, which grows forward from the posterior part of the germ-wall. In speaking of this forward growth he says: "Es gehen nämlich von der Gegend des hinteren Keimwalles, als unmittelbare Fortsetzung desselben, kurze Zellstränge aus, die mitten durch die Keimhöhle nach vorn ziehen, miteinander in Verbindung treten und eine dünne Platte von verschiedener Dicke und vielen grösseren und kleineren Löchern bilden. Diese Platte endet vorn und an den Seiten mit freiem,

wenn auch unregelmässigem Rande, zeigt also ein zungenförmiges Aussehen. Man wird wohl nicht fehlgehen, dieses Gebilde als den Anfang der unteren Keimschicht, d. h. des Entoderms zu bezeichnen. Es ist dies allerdings nicht das einzige zellige Material, was innerhalb der Keimhöhle zu finden ist, aber doch die bei weitem grösste Menge."[1] (3) The third class includes those who believe that the entoderm arises by a process of gastrulation, that is, the upper layer turns under to give rise to the lower layer. This view has been supported by Haeckel, Goette, Rauber, and others. The work of Duval ('84) also has been quoted in support of gastrulation. This author describes the blastoderm at the end of segmentation as a biconvex lens (lentille biconvexe), in which two layers can be recognized ; an upper epithelium-like layer separated by a narrow fissure from a thick lower layer. The deepest cells of the latter are open below to the white yolk of the Nucleus of Pander. In a later stage a thickening occurs on the margin where the upper layer is united with the lower. Duvals calls this thickened rim the *bourrelet blastodermique*. It corresponds to the *Randwulst* of the German authors. At the posterior margin where the rim is thickest, a crescent-shaped groove appears, which passes forward beneath the blastoderm as a fissure separating the lower cells of the blastoderm from the underlying yolk. Duval now regards the blastoderm as in the gastrula stage and hence the fissure between the yolk and the thick lower layer is the archenteron. It is clear that, in the main, Duval's theory is one of delamination. So far as the pigeon's egg is concerned the segmentation cavity is *not found* just below the superficial layer of cells at the end of segmentation, but is situated beneath the central portion of the blastoderm — between the deepest cells and the yolk.

In order to work out the history of a continuous developmental process, such as gastrulation, it is necessary to have a complete series of normal stages taken from one type. Such a series is easily obtainable from the pigeon, and the following account of gastrulation is based upon a study of several series of this birds's egg.

In seeking for a stage at which to begin the account of gastru-

[1] *Loc. cit.*, p. 27.

lation, I found the close of segmentation to be the most advantageous time, for it is shortly after this period that the first direct steps leading up to invagination occur. At the close of segmentation the disc is three or four cells deep, except at the extreme margin where it gradually diminishes to a thickness of one or two cells. Beneath and external to the marginal cells of the disc, yolk or "periblastic" nuclei are present. According to Miss Blount ('07) these nuclei segregate about themselves the neighboring protoplasm, and later, cell walls appearing are added to the disc, thus contributing to its extension. Waldeyer ('69), Hertwig ('99), and others have advanced similar views for the chick and selachian, designating it supplementary cleavage. Where these cells are being added to the disc a more or less syncytial condition exists around the entire margin. This region constitutes the germ-wall.

Shortly after the period described above there occurs the first direct step in the process of gastrulation. This is in the nature of a thinning of the posterior part of the segmented disc. This process begins, not at the extreme margin, but usually slightly posterior to the center, and then spreads in all directions, but with more rapidity towards the posterior margin.[1] The first stage of this process is shown in Fig. 2. Slightly posterior to the center and almost directly above the segmentation cavity, the disc is but two cells deep, while in the region of the germ-wall it is four deep. The characteristic features given above for the germ-wall and the extension of the disc can also be made out from this figure. At this time the segmentation cavity is still very shallow, but upon further progress of the thinning out it becomes much more extensive, and may then be called the subgerminal cavity.

As the thinning out progresses the germ-wall becomes interrupted in the posterior region, as is shown in Fig. 4. The blastoderm from which this drawing was made is much more advanced than that in Fig. 2, being about eleven hours older. The changes occurring between these two stages, however, are gradual and may be followed with comparative ease. In the first place there is a very rapid division of cells, as is evidenced by

[1] *In Torpedo ocellata* Zeigler ('02) describes the thinning-out of the blastoderm as beginning at the posterior and progressing anteriorly.

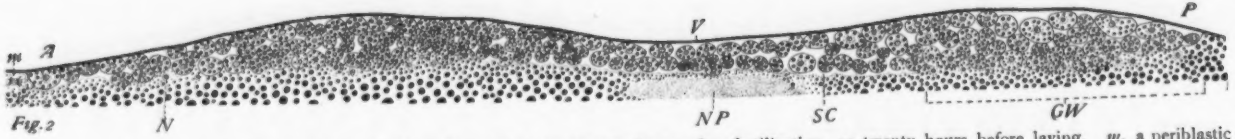

Fig. 2

FIG. 2. A median longitudinal section of a blastoderm taken twenty-one hours after fertilization, or twenty hours before laying. *m*, a periblastic nucleus in the process of division. ×77·3.

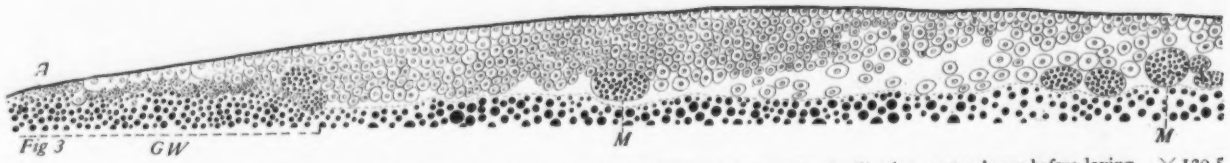

Fig 3

FIG. 3. Anterior half of a median longitudinal section of a blastoderm taken thirty-one hours after fertilization, or ten hours before laying. ×129.5.

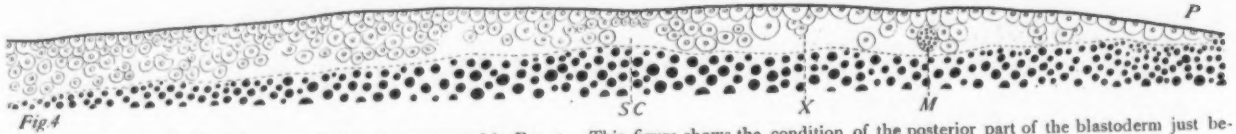

Fig 4

FIG. 4. Posterior half of the same section as represented in FIG. 3. This figure shows the condition of the posterior part of the blastoderm just before invagination begins. *X*, cells crowding into the ectoderm. ×129.5.

their number and size in the anterior half of the blastoderm (Fig. 3), where they are about seven layers deep. However, as one passes from the anterior to the posterior margin there is a gradual change in depth from seven cells to one. There are also found in the anterior region large yolk masses (Fig. 3, *M*), which arise from the floor of the segmentation cavity. At this stage they are not limited to this region, but occasionally are found in the posterior half (Fig. 4, *M*), where the disappearance of the germ-wall is one of the most characteristic features. This interruption of the germ-wall goes hand and hand with the thinning out, which is rapidly establishing a one-layered condition of the blastoderm. In other words the phenomenon of thinning-out is nothing more nor less than the crowding of the cells of the segmented disc into a single layer. It is evident that this must result in a rapid centrifugal expansion of the blastoderm. That this is actually the case is shown by measurements. Thus at twenty hours after fertilization the average diameter of the blastoderm is 1.915 mm., while at thirty hours it is 2.573 mm. In fact there is no other period in the early history of the blastoderm in which there is such a rapid increase in the surface area, as occurs during the time when the thinning out is at its maximum. One would not be justified, however, in saying that this entire expansion is brought about by the thinning out, for according to Miss Blount's interpretation the germ-wall is also contributing materially to this increase.

In the posterior third of this blastoderm the single layer is almost complete; still, at places, some of the few cells yet remaining in the segmentation cavity can be seen apparently in the act of crowding up into the single layer (Fig. 4, *X*). Whether or not, in all cases these remaining cells eventually succeed in getting into the upper layer, approximately above where they are situated is not clear. They do in the majority of blastoderms, but I have some few series in which they seem to migrate anteriorly and to the sides, where the last stages of thinning out occur. In either case they take no part in the formation of the gut-entoderm.

In Fig. 5 is shown a reconstruction from sections of the blastoderm represented in Figs. 3 and 4. The germ-wall does not

completely encircle the rim of the blastoderm, but it is interrupted for a distance of about 70 degrees at the posterior margin. The form of the germ-wall is that of a crescent, with its horns stopping slightly short of the area over which a single layer has been developed.

During the two or three hours immediately following the stage just described, the thinning out continues to extend anteriorly over the central portion of the blastoderm, and at the same time spreads laterally. By the time the posterior third of the blasto-

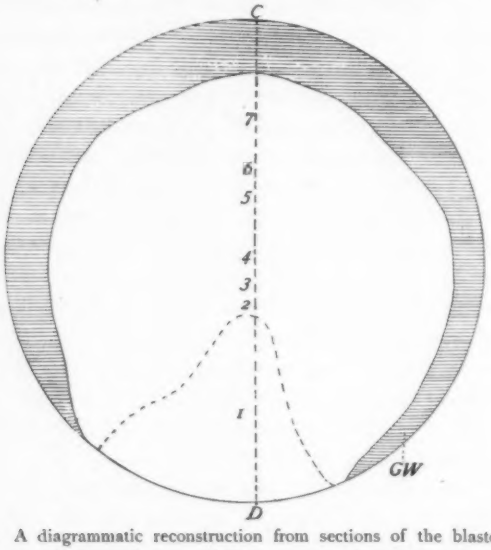

FIG. 5. A diagrammatic reconstruction from sections of the blastoderm from which Figs. 3 and 4 were drawn. *GW*, germ-wall. Numbers 1, 2, 3, etc., represent the regions of the blastoderm which are one, two, three, etc., cells deep, respectively. The broken line around 1 indicates the region where the blastoderm is approximately one cell deep. × 27.2

derm is thinned out to approximately one layer there occurs the initial step in gastrulation. Owing to the individual variation in the development of eggs, some difficulty has been experienced in securing a complete series through this brief period, so that at present I am not in a position to state positively in what this initiatory step consists. The evidence, however, inclines me to believe that it is brought about by a rolling under of the thin

free edge of the posterior margin of the blastoderm. This interpretation is in harmony with what is known to occur in the gastrulation of other forms, especially the fish. Thus Agassiz and Whitman ('84) state that in *Ctenolabrus* "there is a plain rolling under, or involution, as an initiatory step in the formation of the ring," but they believe that it is more correct to describe the process "as an ingrowth, due both to a rapid multiplication of the cells, and also to the centrifugal expansion of the ectoderm." There are certain differences between the teleost and pigeon blastoderms which, in this connection, must not be overlooked. Thus at the time of invagination the teleost blastoderm is three or four cells thick, and the epidermal layer of the ectoderm takes no part in the involution. On the other hand, the pigeon blastoderm is approximately but one cell thick at the posterior margin where invagination occurs, and hence all the cells of this margin participate in the involution. The interpretation of sections certainly supports this view, but the appearance of sections is often misleading. Two other sources of evidence are much more convincing. In the first place, careful measurements show that previous to and following gastrulation the blastoderm is nearly circular, but during the period of invagination the antero-posterior diameter is *always shorter* than the transverse diameter. This is exactly what one would expect if the posterior margin turns under instead of growing out over the yolk. In the second place an injury made on the posterior margin of the blastoderm during the early stages of invagination is found, upon further incubation, in the gut-entoderm, that is, it has been carried down under the blastoderm.

There occurs simultaneously with the turning under of the free edge a rapid thickening in the region of invagination, that is, on the posterior border where the upper layer turns under to become continuous with the invaginated portion. This thickening is not to be accounted for merely by a multiplication of cells *in situ*, but is largely brought about by a movement of material to the median axis from the lateral portions of the posterior margin of the blastoderm. This shifting of material necessarily brings about the approximation of the horns of the germ-wall, and in thus approaching each other they finally meet, and thus close the

blastopore. All this will become clear upon examining a blastoderm during the period in which gastrulation is at its height, and comparing it with a stage such as is shown in Fig. 5.

Fig. 6 is a reconstruction of a blastoderm at the height of gastrulation, which was taken five hours before laying, or thirty-six hours after fertilization. It represents a dorsal view as though the ectoderm were transparent. On the anterior and lateral margins is a clear crescent-shaped area (*O*), the region of over-growth.

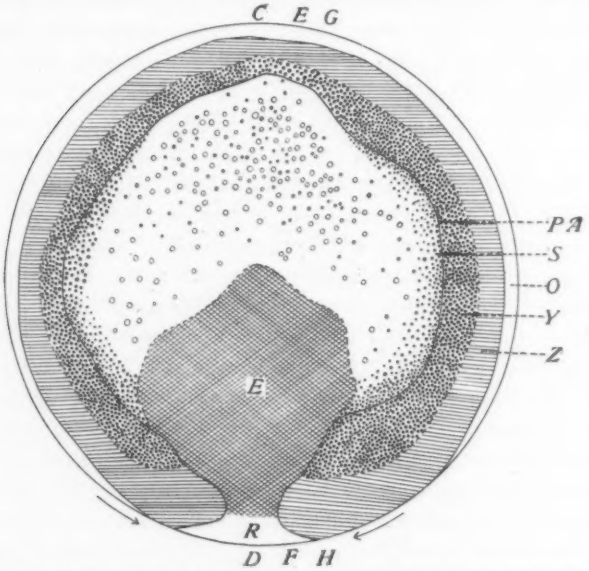

FIG. 6. A diagrammatic reconstruction of a blastoderm taken thirty-six hours after fertilization, or five hours before laying. It represents the ectoderm as transparent. *O*, region of overgrowth; *Z*, zone of junction; *Y*, yolk zone; *PA*, outer boundary of the area pellucida; *S*, beginning of yolk-sac entoderm; *E*, region covered by invaginated or gut-entoderm; *R*, dorsal lip of the blastopore. Lines drawn through *CD*, *EF*, and *GH* represent the planes of the sections illustrated in Figs. 8, 9 and 10, respectively. The arrows at posterior margin indicate the direction of movement of the halves of the margin. × 27.2.

Between this area and the subgerminal cavity (*PA*) is the germ-wall, in which two distinct zones can be recognized. The outer of these (*Z*) may be designated as the *zone of junction*,[1] and is

[1] This term was first used by Agassiz and Whitman, '84.

characterized by a fusion of layers, in which there may be a more or less syncytial condition. Above the region of the inner or *yolk zone* a distinct ectoderm is present, below which are found many large cells heavily laden with yolk. These cells, which may be more or less surrounded by yolk, in which numerous "periblastic" nuclei are present, are destined to become the yolk-sac entoderm. This zone is the region formerly occupied by the zone of junction.

Within the subgerminal cavity are important structures. Scattered over the greater part of its area, but more numerous in the anterior region, are many large yolk masses, among which are also a few of the remaining segmentation cells that have not yet succeeded in getting into the ectoderm. At the extreme sides of the cavity are a great number of cells (*S*), which in the main are the same as the latter. However, it is possible that some of these may have been given off from the inner edge of the germ-wall. *E*, a tongue-like process, is the region over which the invaginated entoderm extends. It reaches from near the posterior margin to slightly beyond the center of the blastoderm. Its anterior and antero-lateral margins end freely, but its postero-lateral margins are bounded by the horns of the germ-wall. At the extreme posterior the entoderm is in connection with the thickened rim, a region of indifferent structure. Where the entoderm arises from the rim it is necessarily thick but it gradually thins out anteriorly. Beneath this rim (*R*) is a passage, the blastopore, which is widest at the margin, gradually narrowing as it passes toward the center. This passage becomes continuous with the cavity under the entoderm — the archenteric cavity.

Sections of this blastoderm are very instructive. A portion of the anterior half of a section, four sections to the left of the median line, is shown in Fig. 7. At the extreme anterior margin is the region of overgrowth (*O*), where the blastoderm first spreads over the yolk. The width of the overgrowth never exceeds that shown in this series, for as fast as it extends out over the yolk the germ-wall keeps pace with it. In the more peripheral portion of the germ-wall separate layers cannot be distinguished. This is the zone of junction, which seldom has a greater width than is shown in this figure. But next to the

subgerminal cavity a distinct ectoderm can be recognized, and just beneath this ectoderm are cells mingled with yolk granules in which periblastic nuclei are present. As the overgrowth proceeds over the yolk, followed by the zone of junction, it is evident that that portion of the germ-wall between the zone of junction and the subgerminal cavity (*SG*), that is, the yolk zone (*Y*), will continue to increase in width. But the cavity, due to the liquefaction of yolk, is also increasing in width, but at a slower rate. In this widening of the cavity there are left around its margin cells which were previously embedded in the yolk. These cells form the beginning of the yolk-sac entoderm, and when the invaginated entoderm has spread over the subgerminal cavity its free margin becomes continuous with that of the yolk-sac entoderm. The last place for this union to occur is in the anterior region of the cavity.

Within this region of the cavity are found many large yolk masses (*M*), in some cases so numerous as to cause an elevation of the ectoderm, especially in later stages. Against the under surface of this layer are crowded a few remaining segmentation cells (*X*). These are the cells which Gasser ('82) has mistaken for wandering entoderm cells and Nowack ('02) for wandering ectoderm cells. At the extreme right of this figure the free end of the invaginated entoderm can be seen (Fig. 7, *L*).

Fig. 8 is a portion of the posterior half of a longitudinal median section (see Fig. 6). In the posterior region is the thickened rim, or dorsal lip of the blastopore (*R*).[1] From its rounded appearance one might infer that the posterior margin is still rolling under to form the entoderm, but at this stage it is more correct to regard the entoderm as arising as a forward growth from the inner edge of the thickened rim. At the place of origin (*U*) the entoderm is very thick, but gradually thins out anteriorly. The blastopore (*B*) is a shallow passage extending inward from the exterior to become continuous with the archenteric cavity (*AC*), which is that portion of the subgerminal cavity covered by the entoderm. Within the archenteric cavity are two large yolk masses (*M*) which have just risen out of the yolk. Below the

[1] The ventral lip is represented by the yolk lying immediately beneath the blastopore.

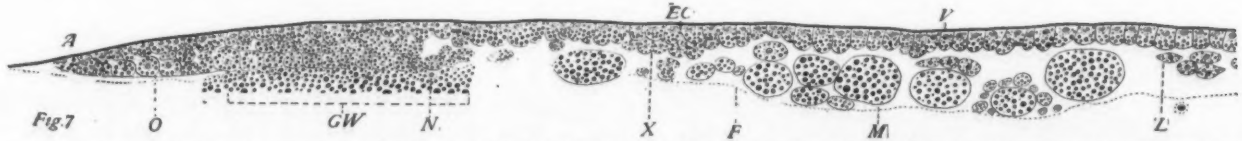

FIG. 7. Anterior portion of a section taken slightly (4 sections) to the left of the plane passing through *CD* of Fig. 6. *X*, segmentation cells crowding into the ectoderm. × 169.

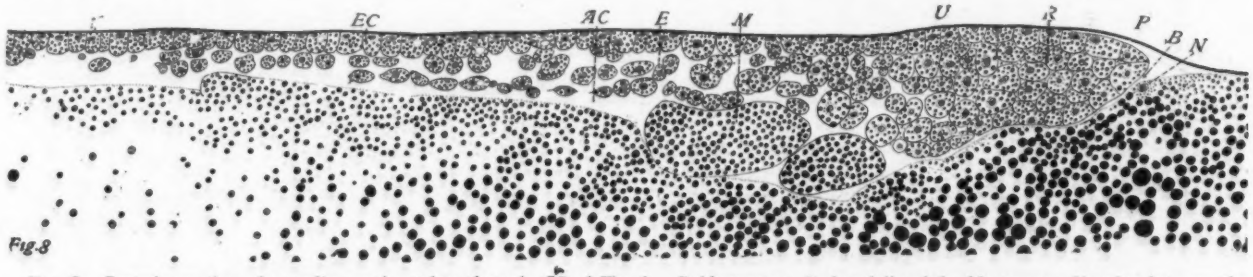

FIG. 8. Posterior portion of a median section taken through *CD* of Fig. 6. *B*, blastopore; *R*, dorsal lip of the blastopore; *U*, union between the deeper cells of the dorsal lip and the entoderm. × 169.

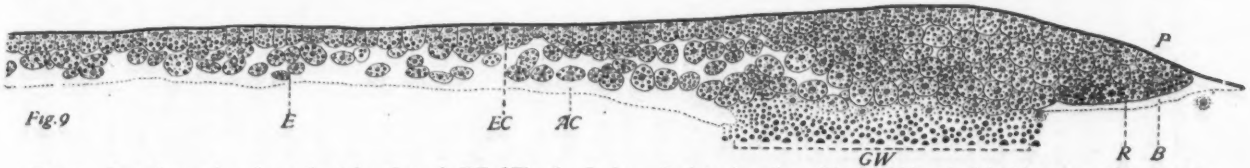

FIG. 9. Posterior portion of a section taken through *EF* of Fig. 6. It shows the lateral portion of the lip (*R*) and of the blastopore (*B*). × 169.

blastopore no periblastic nuclei are present, except a few at the extreme margin of the blastoderm (*N*).

Fig. 9 represents the posterior portion of a section taken through *EF* of Fig. 6. At the extreme right is the dorsal lip of the blastopore (*R*). This lateral part of the lip is not so thick as in the median section (Fig. 8, *R*). Below the lip is the lateral portion of the blastopore. The section also passes through the end of the right horn of the germ-wall (*GW*). The remaining structures are very similar to the corresponding parts of Fig. 8 and need no further description.

From Fig. 6 it will be seen that a section taken through *GH* would no longer contain any portion of the blastopore, since in this region the outer edge of the germ-wall reaches to the margin of the blastoderm. This section is represented in Fig. 10, and its most important part, consisting of a mass of cells from which the entoderm arises anteriorly, is shown at *D*. Between this mass and the inner edge of the germ-wall there is a space in which only a few cells are present. In some sections, however, no such space exists, but the mass of cells is directly continuous with the germ-wall. In such cases it is easy to gain the impression that the mass is a part of the germ-wall, and thus to be led astray into concluding that the entoderm arises directly from the germ-wall. A close study, however, shows that the character of this mass, even in cases of its most intimate union with the germ-wall, is such as to make it easy to distinguish the one from the other. As previously stated, many of the cells in the region of the germ-wall are directly open to the underlying yolk, in which periblastic nuclei are present, but the cells of the mass are entirely separated from the yolk and are completely delimited by cell-walls. The significance of this mass will be considered in connection with Fig. 13.

Fig. 11 is a median longitudinal section of a blastoderm taken thirty-eight hours after fertilization. It shows the condition of the blastoderm shortly after the closing of the blastopore, and clearly represents the character of the entoderm during the few hours immediately following this event. It will be seen that the entoderm is not a continuous layer, especially in the anterior region, where the cells are more or less in groups. Later, how-

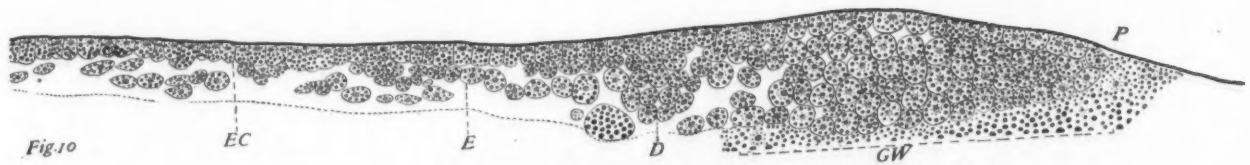

FIG. 10. Posterior portion of a section taken through *GH* of Fig. 6. *D*, mass of cells. × 169.

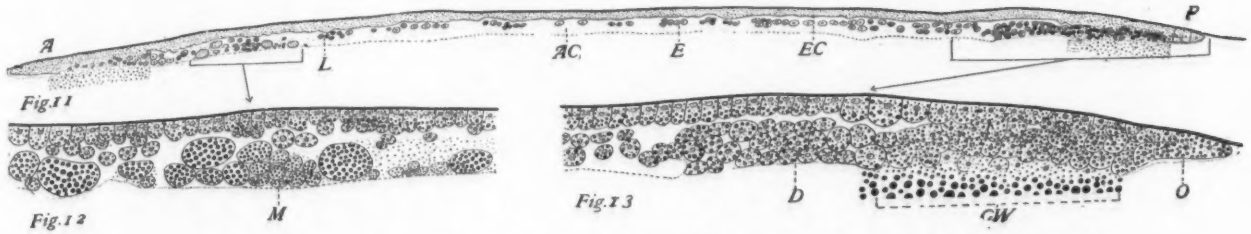

FIG. 11. A median longitudinal section of a blastoderm taken thirty-eight hours after fertilization, or three hours before laying. × 72.
FIG. 12. Enlarged anterior portion of the subgerminal cavity of the section represented in Fig. 11. × 169.
FIG. 13. Enlarged posterior portion of Fig. 11. × 169.

ever, these cells spread out and at the same time become flattened thus filling the intervening spaces and producing the characteristic gut-entoderm. At the stage represented in this figure, the entoderm in its forward growth has not yet reached the anterior limit of the subgerminal cavity, but its free edge ends about .35 mm. from this point (Fig. 11, *L*). The anterior part of the cavity not yet penetrated by the entoderm is occupied mainly by large yolk masses (Fig. 12). In the posterior part of this section the entoderm is directly continuous with the *mass* of cells (Fig. 13, *D*), which in turn is continuous with the inner edge of the germ-wall, and posterior to this wall is the region of overgrowth (*O*). In order to prove the origin and significance of this mass one must have recourse to experimental data. This form of evidence shows that the right and left halves of the dorsal lip of the blastopore grow toward each other and fuse in the median plane, that is, in the plane of the future longitudinal axis of the embryo. This movement of material from the lateral halves is not confined to the dorsal lip alone, but is participated in by the more lateral portions of the margin, that is, by the horns of the germ-wall. In the large majority of blastoderms, however, the right and left horns of the germ-wall do not turn in along the median line and fuse, but their free ends, upon meeting simply coalesce and grow out over the yolk [1] (see Fig. 6). It should also be borne in mind that as the horns are moving toward the median line, they are at the time being carried centrifugally by the expansion of the blastoderm. In this way the fused halves of the blastoporic lip are enclosed just anterior to the inner edge of the germ-wall, and hence the mass of cells, referred to above, is derived from the deeper portions of this enclosed lip. This is most apparent immediately after the ends of the horns have met, when the ectoderm is not yet differentiated from the underlying mass.

The movement of the two lateral halves of the posterior margin toward the median line and their simultaneous fusion must be regarded as a form of "concrescence"—the right and left halves

[1] In the cases in which a "marginal notch" is present, and in the rare cases such as that described by Whitman ('83) the horns of the germ-wall must also turn in and fuse.

of the dorsal lip representing the "homotypical" halves of the future embryo.[1]

A reconstruction of the blastoderm represented in Figs. 11–13 is shown in Fig. 14. The over-growth (*O*) and both zones of the germ-wall (*Y* and *Z*) completely encircle the blastoderm. Just anterior to the inner edge of the posterior germ-wall is the mass of cells (*R*) which is thick in the center, but gradually thins out

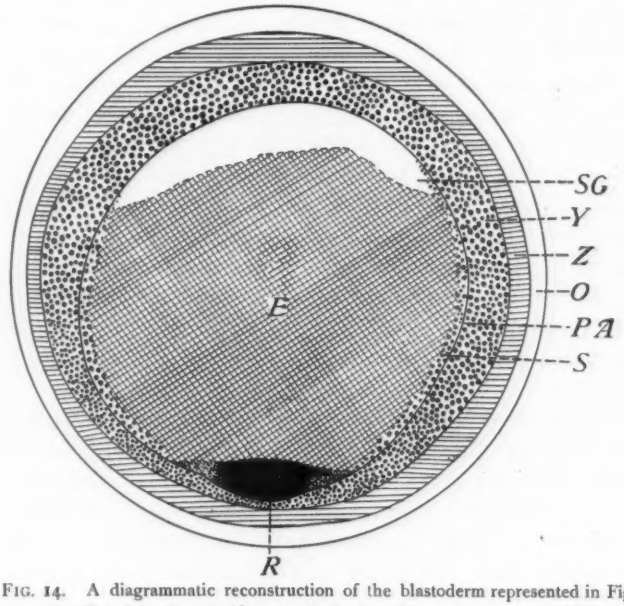

FIG. 14. A diagrammatic reconstruction of the blastoderm represented in Figs. 11–13. See Fig. 6 for the significance of the lettering. *R*, mass of cells, or the deeper cells of fused halves of the dorsal lip. × 27.2.

towards the sides.[2] Anteriorly it is continuous with the entoderm, into which it is differentiating as the latter grows forward through the subgerminal cavity. At this stage the entoderm (*E*) still ends with a free edge anteriorly, but its lateral margins are united with the yolk-sac entoderm at *S*. The entoderm does

[1] This view does not differ essentially from that advanced for the chick by Rauber ('76), Whitman ('78 and '83) and others — a view hitherto not supported by experimental workers, mainly because they experimented upon stages which were too far advanced.

[2] This mass probably corresponds to what Koller ('81) called a "Sichel."

not reach the anterior limit of the subgerminal cavity until from two to four hours after laying, at which time the mass disappears.

Origin of the Primitive Streak.

The primitive streak in the pigeon's blastoderm becomes visible in surface views between the fourth and fifth hours of incubation. In sections, however, it can be detected as early as two hours previous to this time. Its first appearance in section is that of small protuberances of cells on the under surface of the ectoderm situated along the median line. In their longitudinal extension these swellings reach from the posterior edge of the *area pellucida* to a point lying about half way between this edge and the center of the blastoderm (Fig. 15, *ps*). Under high magnification (Fig. 17, *ps*) these swellings are seen to be groups of rapidly dividing cells, which at first are separated from the gut-entoderm, but upon further growth come in contact with it. At the stage represented in Fig. 17 the gut-entoderm is a single layer of flattened cells and is directly continuous with the yolk-sac entoderm (Figs. 15–17, *Y*). The latter is thicker just posterior to the primitive streak than in any other region of the yolk zone.

The evidence afforded by a study of gastrulation indicates the line along which one must look for an explanation of the origin of the primitive streak. During the progress of concrescence there are laid down along the sides of the future longitudinal axis of the embryo strips of primary ectoderm, which are fused along the median line. These strips were previously the superficial cells of the right and left halves of the dorsal lip of the blastopore. For four or five hours after the closing of the blastopore, this median strip cannot be distinguished from the adjoining ectoderm. It is not until the protuberances begin to make their appearance that any difference can be seen, and even then, the double structure of the median region is not evident. In fact, it is only when the primitive groove appears that this bilateral structure becomes clear.

In conclusion, I may say, that during the process of gastrulation only the gut-entoderm is involuted; the chorda and mesoderm arise from the primitive streak, which represents the fused

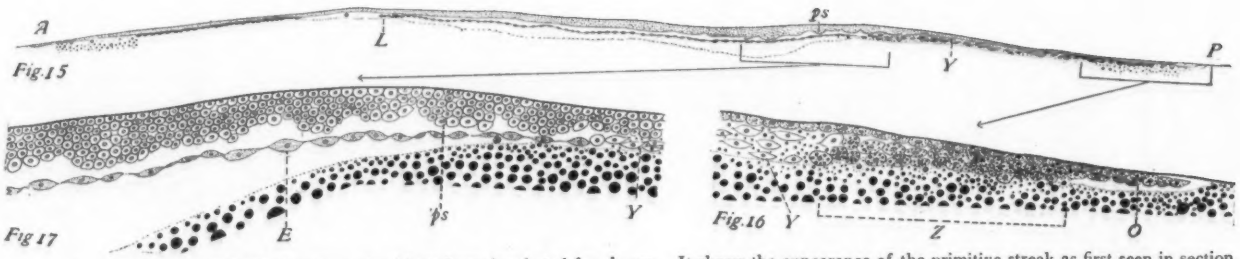

FIG. 15. A median longitudinal section of a blastoderm incubated four hours. It shows the appearance of the primitive streak as first seen in section. ×41.

FIG. 16. Enlarged portion of the posterior end of Fig. 15. ×169.

FIG. 17. Enlarged portion of the primitive streak region of Fig. 15. ×169.

halves of the 'dorsal lip of the blastopore. The main evidence in support of this view is derived from experimental data, which will be presented in a future paper.

It gives me pleasure here to acknowledge my indebtedness to Professor Whitman, under whose direction the work has been carried on, and also to Professor F. R. Lillie for help and criticism.

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July 10, 1907.

COMMON REFERENCE LETTERS USED IN THE FIGURES.

A Anterior end of the blastoderm.
AC Archenteric cavity.
B Blastopore.
E Invaginated or gut-entoderm.
EC Ectoderm.
F Floor of the subgerminal cavity.
GW Germ-wall.
L Anterior limit of the gut-entoderm.
M Yolk masses.
N Periblastic nuclei.
O Region of overgrowth.
P Posterior end of the blastoderm.
R Dorsal lip of the blastopore.
SC Segmentation cavity.
SG Subgerminal cavity.
V Vitelline membrane.
Y Yolk zone.
Z Zone of junction.

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THE EFFECTS OF SALTS AND SUGAR SOLUTIONS ON THE DEVELOPMENT OF THE FROG'S EGG.

T. H. MORGAN AND C. R. STOCKARD.

The purpose of these experiments was to determine more definitely what action takes place when eggs of the frog are treated with solutions containing both salts and sugar, as compared with control solutions containing only salt or sugar. We have also tried to compare the action of such sugars as cane sugar, that might possibly invert, with the action of simpler sugars, like glucose or lævulose. We have kept in mind the possibility that while the chief action of sugar is through its osmotic pressure, yet sugar may also act chemically on the living substance; or by forming new compounds with the inorganic salts may affect the results in this way.

Action of Lithium Chlorid and Sodium Chlorid Acting Alone.

The concentration of LiCl that will prevent the development from proceeding beyond the segmentation stages was previously determined for the egg of *Rana sylvatica* to be about 0.65 per cent. Since the salts obtained at different times may vary in the amount of absorbed water, it was necessary (for percentage solutions were used) to make a new determination as control for exact comparisons with the effects of the new salt when united in solution with sugars. The eggs were put into the solutions in the 2-cell stages in all cases. It was found that in LiCl 0.5 per cent. (the strength used in combination with sugar) the blastopore appeared and sometimes closed almost normally; usually it formed a ring around or below the equator of the egg. Lithium chlorid of this strength is, therefore, near the limit of inhibitory effects but below that limit. Some solutions of this strength were used with sugar solutions.

The upper limit of NaCl was not accurately determined in previous work on the frog[1] and the results are not in harmony with

[1] Morgan, T. H., "Experiments with Frog's Egg," Biol. Bull., XI., 2, 1906.

the present ones. In NaCl 0.5 per cent. development was nearly normal; in 1.0 per cent. only the 16- and 32-cell stages were reached; in 1.5 per cent. only the 16-cell stage, and in 2.0 per cent. only the 8-cell stage, and in the 2.5 per cent. only the 4- or 8-cell stage was reached, while in the 3.0 per cent. the eggs died without developing further. Similar results were obtained in another series of the same kind. In a third series of smaller range it was found that in a 0.5 per cent. solution the later cleavage stages were reached; in a 0.7 per cent. solution also only the late cleavage stages appeared; in a 0.9 per cent. solution the cleavage had not gone so far, while in a 1.0 per cent. solution only the 32- or 64-cell stages had been formed.

The upper limit for NaCl lies, therefore, somewhere between 0.5 and 1.0 per cent. and not above 2.0 per cent. as previously stated. This difference in the results may possibly have been due to some impurities in the NaCl, not present in the salt used the previous year. The osmotic pressure for 0.5 per cent. is 3.55 atmospheres and for 1.0 it is 6.96. The latter is above that of LiCl 0.65 per cent., which is 6.16.

Action of Solutions Containing both Salts and Sugars.

Previous work on the frog's egg had indicated that when to a salt solution, too weak in itself to prevent development, a certain amount of sugar is added the development may be prevented; and a comparison of the osmotic pressures showed that in such a solution the pressure is higher than that when the salt alone produces the same results, but lower than that necessary for the sugar alone to produce the effect. Similar results have been obtained for the salt-water fish, *Fundulus*, where the outcome is even more striking owing to the fact that the eggs of this fish will not develop in a fresh water solution of salt and sugar that has an osmotic pressure lower than that of sea water in which they normally develop.[1] We have gone over these results with the frog in order to make sure that the effects were not due to the inversion of the cane sugar previously employed (that would increase its osmotic pressure) or that the effects were not due to an adulteration of the cane sugar with other sugars.

[1] Stockard, C. R., "The Influence of External Factors, Chemical and Physical, on the Development of Fundulus heteroclitus," *Jour. Exp. Zoöl.*, IV., 2, 1907.

In a ten per cent. solution of cane sugar the blastopore may develop, but in a thirteen per cent. solution only the later cleavage is reached. In a 5.5 per cent. solution of glucose the blastopore may develop in an abnormal way, while in a 6.0 per cent. solution only the late cleavage stage is reached. The results show that the same effect is produced by the same osmotic pressure of the two sugars.

A double solution of LiCl 0.5 per cent. plus glucose 0.5, 0.7, and 1.0 per cent. gave the following results:

LiCl 0.5 per cent. plus glucose 0.5 per cent., late segmentation.
LiCl 0.5 per cent. plus glucose 0.7 per cent., late segmentation.
LiCl 0.5 per cent. plus glucose 1.0 per cent., segmentation more abnormal.

The action of LiCl 0.4 per cent. plus glucose 1.0, 2.0 and 2.5 per cent. was as follows:

LiCl 0.4 per cent. plus glucose 1.0 per cent., very late segmentation: abnormal blastopore.
LiCl 0.4 per cent. plus glucose 2.0 per cent., late segmentation; not so far.
LiCl 0.4 per cent. plus glucose 2.5 per cent., late segmentation.

The results show that by adding amounts of glucose to a solution of LiCl, the development is stopped at a pressure higher than that for LiCl alone, but less than that for glucose alone.

Another similar experiment gave the same results. In a third experiment with LiCl 0.4 per cent. plus glucose 0.5, 1.0, 1.5 and 2.0 per cent. the results were nearly the same as is shown below.

LiCl 0.4 per cent. plus glucose 0.5 per cent., gastrulation normal, but delayed.
LiCl 0.4 per cent. plus glucose 1.0 per cent., barely gastrulating: abnormal.
LiCl 0.4 per cent. plus glucose 1.5 per cent., barely gastrulating: abnormal.
LiCl 0.4 per cent. plus glucose 2.0 per cent., late segmentation only.

The upper limit for this combination is about LiCl 0.4 per cent. plus glucose 2.0 per cent. with an osmotic pressure of 6.63, which is above LiCl .65 (= 6.16), but lower than glucose 6.0 (= 8.376).

The results with NaCl plus glucose were as follows: In a solution of 0.5 NaCl plus glucose 1.0, 1.5, 2.0 and 3.0 per cent. the late segmentation stages developed, but the yolk was injured. In another experiment the results were more decisive.

NaCl 0.5 per cent. plus glucose 1.0 per cent., circular blastopore above equator.
NaCl 0.5 per cent. plus glucose 1.5 per cent., late segmentation.
NaCl 0.5 per cent. plus glucose 2.0 per cent., dead in segmentation stages.
NaCl 0.5 per cent. plus glucose 3.0 per cent., not so late segmentation.

The upper limit for this combination lies therefore about NaCl 0.5 per cent. plus 3.0 per cent. glucose.

The Action of Sugar Solutions.

The experiments with sugar solutions were conducted to find if possible an explanation of the peculiar results which Stockard had obtained during the past summer by treating *Fundulus* eggs with solutions of sugars and sugar and salt mixtures. When *Fundulus* eggs were subjected to a solution containing LiCl or NH_4Cl and cane sugar the action of the salt was greatly augmented by the presence of the sugar even though the osmotic pressure of the mixture was lower than that of sea water. This indicated that the more marked action was not due to any increase in osmotic pressure that may have resulted from the addition of the sugar, but to some further or new chemical action.

Equal amounts of sugar were found to exert a more injurious effect on *Fundulus* eggs when in fresh water than when in sea water, although obviously the osmotic pressure of the latter was much the greater. This seemed possibly to indicate that the cane sugar in the fresh water solutions had become inverted, thus producing these pecular results. From a consideration of the experiments below it would seem more probable, however, that sugar exerted some chemical action on the compounds of the egg when in fresh water solutions rather than that inversion had taken place resulting only in an increase of pressure.

Frog eggs when in the four-cell stage were subjected to the following solutions of cane sugar: 6, 8, 9, 10, 11, 12, 12.5, 13, 15, 17 and 20 per cent. Although this is a series of fairly wide range it was found that the eggs were only slightly affected in the 6 per cent. solution, while they reached a late segmentation stage even in the 15 per cent. solution; the limit of effectiveness or fatal dose of sugar is thus seen not sharply indicated as in the case of many salts where a small fraction of a per cent. difference in the concentration of the solution gives at the critical points a marked difference in the effects on the eggs. The specific gravity of the sugar solutions was so high in most cases that the eggs would float in an indifferent position, the greater weight of the yolk pole not serving as it normally does to orient the egg in a definite

manner. The sugar solution was freshly prepared before starting the experiments, and Heines' solution was used to test the sugar to further assure ourselves of its purity and uninverted condition.

The 6 per cent. cane sugar solution delayed the rate of development after about twenty hours, so that when forty-eight hours old the eggs were far behind the control; and although neural folds and other indications of the embryo were present the embryonic outline was usually shortened as an effect of the delayed blastopore closure. The 8 per cent. solution gave much more marked effects. After twenty-four hours abnormal gastrulæ were formed, though none became elongated or showed any indication of embryo formation. Such a condition is similiar to that described below for eggs in 4 and 5 per cent. solutions of glucose and lævulose; the pressures of the 5 per cent. solutions are, however, slightly more than that of the 8 per cent. cane sugar. After fifty hours all of the eggs in the 8 per cent. solution were dead. Cane sugar of 9 per cent. had much the same effect.

Solutions of 10, 11, 12 and 12.5 per cent. cane sugar gave rather uniform results. Development was delayed within ten hours or less and usually stopped before gastrulation had commenced. In the 10 per cent. solutions, however, some eggs formed very abnormal gastrulæ of a rather uniform type, the upper dark or micromere portion of the egg had sunken in the lower coarser cells suggesting somewhat in gross appearance an acorn held in its saucer-like burr. It is of interest to note that such a type of gastrula was also found in the 5 per cent. glucose and lævulose solutions which exert approximately the same pressure as the 10 per cent. cane sugar.

The 13 and 15 per cent. solutions act much the same, the weaker giving less marked effects than the stronger one. After ten hours the eggs were much delayed, the white area had not been encroached upon by the darker cells and was divided into only six or eight large blastomeres. The eggs were all much plasmolized and development after twenty-two hours in the solution had progressed only about as far as control eggs of nine or ten hours old. Late segmentation was reached, and the eggs died in this condition after forty-five hours.

The 17 per cent. sugar exerts a pressure of about 11.86 atmospheres above that of the normal medium in which these eggs live. The eggs were effected very readily under such conditions. After only six hours in the solution a few showed no cellular structure at all, while the others were far behind the control in their rate of development. All died in late segmentation stages.

The 20 per cent. cane sugar stopped the development of most eggs within six hours, the blastomeres seemed to have fused together. A very few eggs divided to about the sixth or seventh cleavage and then underwent cytolysis.

Lactose or milk sugar was tried but this substance dissolved so slowly that it was difficult to interpret the results, and since the maximum pressure of the solution was reached only after the eggs had developed much beyond the 4-cell stage, the effects are not readily compared with those resulting from the use of the other sugars.

Simple sugars, glucose and lævulose, which are the inversion products of the cane sugar molecule were tried in order to determine if possible whether solutions of these which were isotonic with a given cane sugar solution would give similar results. Approximate comparisons of these solutions may be made as follows: A 5 per cent. solution of glucose or lævulose exerts a pressure nearly the same as a 10 per cent. solution of cane sugar. It is not exactly the same since a molecule of cane sugar, $C_{12}H_{22}O_{11}$, is a little less than twice a molecule of glucose, $C_6H_{12}O_6$, and in addition to this it must also be borne in mind that equi-molecular solutions of glucose and cane sugar do not exert exactly the same osmotic pressures, although for general purposes the two are considered about equal.

Eggs when in the four-cell stage were placed in the following strengths of glucose, 2, 3, 3.5, 4, 5, 5.5, 5.8, 6, 6.5, 10 and 15 per cent. The 2 per cent. solution had a very weak action causing the development to proceed slower than usual. After forty nine hours in the solution many of the eggs were slightly abnormal.

The 3, 3.5 and 4 per cent. solutions retard development considerably within twenty hours, and those in the 4 per cent. solution show abnormal gastrulation with prominent yolk-hernias.

The eggs in the latter solution are also plasmolized. In all three solutions the eggs died after about fifty hours as abnormal gastrulæ.

Eggs in 5 per cent. glucose in one of the experiments, formed abnormal gastrulæ, which closely resembled those described above in the 10 per cent. cane sugar, but in other experiments only a few eggs attempted gastrulation, and the majority died while in late segmentation stages. In the 5.5, 5.8 and 6 per cent. solutions late segmentation was reached, but all eggs were badly plasmolized with the animal pole flattened. Eggs in a 6.5 per cent. solution died in much the same condition, though plasmolysis occurred earlier in this solution.

Eggs underwent only a few divisions after being subjected to the 10 per cent. glucose solution, the effect was much the same as that of the 20 per cent. cane sugar.

In the 15 per cent. glucose the eggs were readily killed, the blastomeres being ruptured after only one or two divisions, or within about one hour after being subjected to the solution. This serves to convey some idea of how quickly these high osmotic pressures produce an effect.

Lævulose solutions of 2, 3, 5, 5.5, 6, 6.5, 8 and 10 per cent, were used. The general effects of such solutions were almost identical with those described for the same percentage solutions of glucose, showing that the actions were in the main part due alone to their osmotic pressures, and not to any difference in chemical action which the sugars might have exerted. One would not expect the chemical action of these sugars to be marked even if it was at all perceptible.

A consideration of the responses of frog's eggs to sugars would seem to indicate that the more violent action on *Fundulus* eggs of fresh-water solutions of cane sugar when compared with sea-water solutions is not due to the sugar in the fresh water having become inverted. It will be recalled that *Fundulus* eggs are more susceptible to the same percentage solution of a salt in fresh water than in sea water. These facts together with the case before mentioned of the augmented effect produced when sugar is added to a weak solution of a salt in fresh water, even though the pressure of the solution is below that of sea water, go to

show that the results with these eggs are not due so largely to osmotic pressure, but more to some chemical action which appears to take place between the constituents of the egg substance and sugars or salts when contained in fresh-water solutions. It may be that something present in the usual medium in which they develop, the sea water, prevents to some extent such an action, thus both salts and sugar act less violently when applied in sea-water solutions. The physiological condition of the egg may be weakened in fresh water as is indicated by its slightly retarded development in this medium, and under such circumstances they may be more susceptible to external injurious influences.

It is possible that new substances may be formed when sugar is added to salt solutions since some salts, *e. g.*, NaCl may form sodium-sugar compounds. Such compounds might be more or even less toxic than the original chemicals from which they resulted. These suggestions, which are at best speculative, serve to show how far we are from an understanding of the manner in which eggs respond to chemical stimuli, and indicate the importance of obtaining more complete data on the subject.

ZOÖLOGICAL LABORATORY,
COLUMBIA UNIVERSITY, May, 1907.

THE SEGMENTAL ORGAN OF PODARKE OBSCURA.

LOUISE HOYT GREGORY.

In 1892 Goodrich[1] described "A New organ in the Lycoridea." A ciliated organ found on the dorsal side of the body, paired in every segment except the first and last few. The nephridium was found to be a well-developed organ, opening into the body cavity by a ciliated nephrostome. The narrow and tortuous character of the nephridial tube made it impossible to conceive of its acting as a genital duct considering the large size of the eggs. A comparison of the dorsal ciliated organ with the genital funnels of the capitellids described by Eisig — however, suggested the idea that the dorsal ciliated organ might function as a genital funnel. Goodrich could discover no external pore, nor was he able to observe the discharge of the genital products. Yet he considered it possible that the external pore might be formed only at maturity.

In the spring of 1905, Professor Wilson suggested that I examine this question by the study of sexually mature individuals of *Nereis* and at the same time work out the method of the discharge of its sex products. *Heteronereis* material, found in abundance at Woods Hole, was collected in the summers of 1905 and 1906 but no definite result was obtained on this form. In the case of *Podarke obscura*, however, I had better success, and although the results conform in general to the work of Goodrich on other members of the family Hesionidæ, yet they may be sufficiently different in detail to warrant their description.

The material was collected in July and August, 1905, and in June and July, 1906. Mature females were fixed before the time of discharge (which takes place regularly between 7–9 p. m.),[2] during the period of discharge, and after the eggs had been passed out from the body. Immature specimens, collected in

[1] Goodrich, E. S., "On a New Organ in the Lycoridea and on the Nephridia in *Nereis diversicolæ*," *Quarterly Journal of Microscopical Anatomy*, 1892–3, vol. 34.

[2] Treadwell, A. L., "Cytogeny of *Podarke obscura*," *Journal of Morphology*, 1900–1, vol. 17.

June were fixed as well as males obtained throughout the season. Flemming's fluid and sublimate-acetic were found to be the best fixatives. The material was then sectioned and stained in iron hæmatoxylin.

I wish to thank Professor Wilson who suggested the work and under whose direction it has been completed. Thanks are also due Professor Treadwell and Dr. McGregor for their aid in collecting material.

The nephridia of *Podarke* are present in pairs in every segment of the body except the first few. A general cross-section of the body (as is seen in Fig. 1) shows a fairly typical annelid struc-

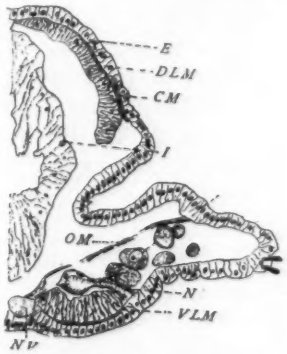

FIG. 1. × 115. A partly diagrammatic transverse section of body. *E*, ectoderm; *D.L.M*, dorsal longitudinal muscle; *C.M*, circular muscle; *I*, intestine; *O.M*, oblique muscle; *V.L.M*, ventral longitudinal muscle; *N.v*, nerve; *N*, nephridium. (The inner end corresponds to the cavity seen in Figs. 2 and 3.)

ture, *i. e.*, the outer layer of epithelial cells, a thin layer of circular muscles, and two pairs only of longitudinal muscles, a ventral and a dorsal pair. In such a section the nephridium is found following the dorsal surface of the ventral longitudinal muscle band. It is a simple tube with practically no convolutions, opening to the exterior laterally on the ventral surface, at outer limit of the ventral longitudinal muscles. (This opening is definitely shown in Fig. 4, the transverse section did not pass through the opening.) The nephridium extends along the upper surface of the muscle, then bends diagonally inward in the segment toward the median plane and forward toward the anterior dissepiment

where it unites with a large ciliated organ in the next anterior segment. (This union is shown in Figs. 2 and 3.) The walls of the nephridium are thin, its cells contain numerous excretory particles and its cavity is lined with fine cilia. The organ receives its blood supply from branches of a ventral longitudinal blood vessel. The position of the blood vessel is shown in the sagittal section, Fig. 2. The organ as a whole is simple in structure and may be regarded as a more or less degenerate condition of the nephridium as seen in *Hesione pantherina*, the nephrostome in the latter being replaced by the ciliated organ.

FIG. 2. × 500. A sagittal section of the segmental organ. Showing the relationship between the ciliated organ and nephridium. *C.O*, ciliated organ; *S*, septum; *N*, nephridium; *B.V*, blood vessel.

The ciliated organs of *Podarke*, like the nephridia, are paired in every segment behind the pharynx. The organ is a thin, flat, more or less triangular plate of ciliated cells, one layer in thickness, which stretches out into the body cavity anteriorly away from the dissepiment with the distal edge of the organ, or the base of the triangle turned toward the intestine and the apex of the triangular mass attached to the nephridium. The dorsal edge of the flat plate is rolled over toward the dissepiment. At the posterior edge it is often compressed and folded, but gradually broadens out into a large lip at the distal end. The ventral portion is only slightly rolled and in this case the turn is away from

the dissepiment. This fold is present only at the distal anterior end of the ventral edge where it forms a short lip.

Immature specimens show the ciliated organ lying directly on the dissepiment, from the cells of the peritoneal covering of which it develops. In mature forms the organ does not lie against the dissepiment except at the point of union with the nephridium. In a sagittal section (Fig. 2) the organ is raised from the dissepiment. Although consisting of only one layer of cells, it may be folded in such a way as to give the appearance of more than one layer. This condition is seen in Fig. 2; if it were not compressed, it would have the appearance of the organ as seen in Fig. 3.

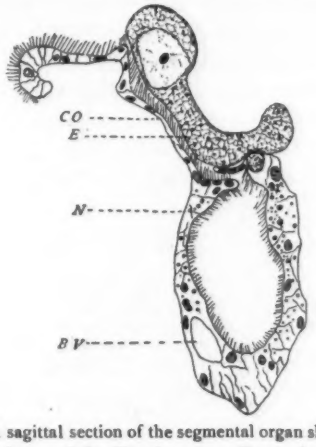

FIG. 3. × 500. A sagittal section of the segmental organ showing an egg passing along the ciliated surface. *C. O*, ciliated organ; *E*, ovarian egg; *N*, nephridial sac; *B. V*, blood vessel.

The ventral lip is not shown in either of the sagittal sections as they passed through the union of the nephridial tube and ciliated organ, whereas the ventral lip which is short and found only at the anterior edge (as has been stated), would appear in earlier sections in the series.

I have not found any indication of an internal termination of the nephridium other than the ciliated organ, and it seems evident that the latter forms an open cœlomic funnel. Its nature may best be considered after an examination of its relation to the discharge of the sex products.

In commencing the work, the material at first showed the nephridia to be so small that it seemed impossible that the large eggs could pass out through them. Goodrich's supposition that the eggs might pass to the outside through a break in the body seemed at first to be confirmed in some of my sections. I found cases where there appeared to be a definite rupture in the center of the ventral longitudinal muscles. This interpretation, however, was proved false, first by the fact that the eggs passing through the rupture were immature, ovarian eggs, whereas, in general, the eggs have been found to form their first polar spindle before leaving the body, and second, by the discovery of eggs passing along the ciliated organ and down into the nephridial cavity (Figs. 3 and 4). The walls of the nephridium are thin, but elastic and are capable of great expansion at the time of maturity. The inner end of the tube swells as the eggs become matured.

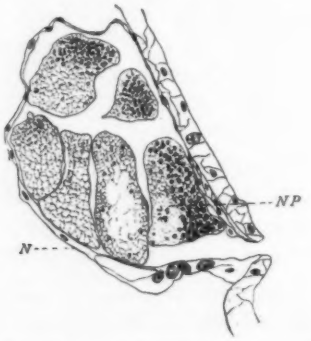

FIG. 4. × 500. A sagittal section of the nephridial sac showing the external opening at the lower end. *N*, nephridial sac; *N.P*, nuclear plate showing that the first maturation spindles are formed before the eggs are extruded. All of the eggs are in the same stage of development, the nuclear plates being visible in different sections.

(The beginning of this is shown in the nephridium in Fig. 1.) Finally the whole tube is distended and has the appearance of a large irregular sac filled with sexual products (Fig. 4). The eggs may pass into the nephridium before forming the polar spindles which are formed in that case, while the eggs are in the nephridial sac. This was probably the case in Fig. 4, or as has been

observed by Treadwell,[1] the spindles may be formed after the eggs have passed out of the body; but, as a rule, the first spindle appears while the egg is free in the body cavity, before its passage into the nephridial cavity. In the male, the relations between the nephridium and ciliated organ is the same as that in the female, but the discharge of the spermatozoa was not observed.

Goodrich[2] in his summary of the most important facts resulting from his study of the Hesionidæ, says: *Hesione sicula* has a nephridium, which opens ventrally to the exterior, passes inwards and forwards, becomes considerably coiled, and finally ends just in front of the intersegmental region by a small, simple, funnel opening into the cœlome of the next segment. Connected with the lip of the nephrostome by a narrow strip of epithelium is a large crescentic genital funnel (ciliated organ), the ciliated surface of which is marked by deep grooves. Those of the middle region converge towards the loose extremity of the organ, where it is connected with the nephrostome and with the body wall. The exact mode of exit of the genital products is unknown.

In *Tyrrhena*, the nephridium is essentially the same but the genital funnel is smaller and more closely connected with the nephrostome.

In *Kefersteinia* and *Ophiodromus* it completely surrounds the inner extremity of the nephridium.

Finally in *Irma*, where the nephridium is no longer coiled, the large genital funnel surrounds and fuses completely with its inner end, forming a trumpet-shaped cœlomic funnel. The genital products, collected together by the action of its ciliated surface pass down into the nephridium and so to the exterior by the nephridiopores.

Podarke seems to be most similar to *Irma*. In both cases, the discharge of the genital products has been determined definitely to be by means of the ciliated organ and nephridium. In *Irma*, however, immature forms show the ciliated organ developing separately from the nephridium, the union taking place when the animal is sexually mature. In *Podarke* immature forms show the

[1] Treadwell, A. L., "Cytogeny of *Podarke obscura*," *Journal of Morphology*, 1900–1, Vol. 17.

[2] Goodrich, E. S., "On the Nephridia of the Polychæta," *Quarterly Journal of Morphology*, vol. 43, 1900.

ciliated organ and nephridium developing in union with one another. The form of the ciliated organ is also quite different from that of *Irma*. There is no resemblance to a "trumpet-shaped organ," the organ being in the form of a flat, triangular mass, as has already been stated, and is united at one point only, not entirely surrounding the nephridium as in *Irma*, yet the rolling of the dorsal and ventral edges may indicate an incomplete funnel.

Fage[1] has worked on these segmental organs in a number of forms in this family, and has found that in *Ophiodromus flexuosus*, *Oxydromus propinquus*, *Kefersteinia cirrata*, there is found a simple nephridium, slightly convoluted, opening to the exterior by a pore found in the region at the base of the parapodium, and into the body cavity by a straight nephrostome. At the moment of sexual maturity a ciliated organ formed from the peritoneum, is united with the nephridium and the sex products pass out through the compound organ. Goodrich did not observe this fact while working on the same forms.

In *Hesione pantherina*, the segmental organ is different, the excretory tube has many convolutions, the nephrostome is well developed possessing long cilia, the entire organ is much more highly modified than in other members of the family, and it closely resembles the excretory organ of the Lycoridea. At the time of sexual maturity, the organ undergoes no transformation. A ciliated organ homologous with that described in other forms of this group, is found near the nephrostome. It resembles the dorsal ciliated organ of *Nereis*. This organ develops at the same time as the nephridium and persists as an independent structure throughout the life of the individual. At its base is found the so-called phagocyte organ, containing in its meshes, granules comparable to the amœbocytes of the cœlome.

Thus we see that when the nephridium is highly developed and adapted more perfectly to its excretory function, it becomes more and more useless as a genital duct, and we find a special genital funnel appearing independent of the nephridium, and serving as a means of discharge for the sexual products. By this form, the family of Hesionidæ is closely connected with the family of Nereidæ.

[1] Fage, Louis, "Organes segmentaires des annelides Polychetes," *Annales des Sciences Naturelles*, Tome III., 1906.

Podarke obscura has been shown to possess a simple, uncoiled nephridial tube with a well differentiated ciliated organ developing at the same time and probably in union with the nephridium. No trace of a phagocyte organ was observed.

As a result of the investigations in this one family of the Polychætes, we may divide the Hesionidæ into three groups.

This classification is modeled somewhat after that made by Goodrich for the whole group of Polychæta. The nephridium has always an internal opening. Solenocytes have not been observed.

Group 1. — Forms in which the segmental organs consists of a nephridium highly differentiated, having a coiled tube and a well-developed nephrostome, and a distinct independent ciliated organ or genital funnel with an external opening through which the eggs are discharged. *Hesione pantherina.*

Group 2. — Forms in which the nephridium is a more simple tube opening into the cœlome by a small nephrostome. At the time of reproduction a ciliated organ is grafted on to the tube and affords a means of exit for the genital products. *Ophiodromus flexuosus, Oxydromus propinquus, Kefersteinia cirrata.*

Group 3. — Forms in which the nephridium is still more simple, having no coils. The nephrostome has been finally lost and its place taken by the ciliated organ or genital funnel, which develops at the same time, and probably in union with the nephridium. In this one group we may find forms illustrating the gradual loss of the nephrostome and the final fusion of the ciliated organ. *Hesione sicula, Tyrrhena, Irma, Podarke obscura.*

In this classification we have a gradual degeneration or modification of the nephridium from a condition where it performs its excretory function only, through a condition where it has become adapted temporarily to the secondary function of offering a means of sexual discharge to a condition finally where it has become so modified that from an early stage, if not throughout life, it functions as an excretory duct and a genital tube.

COLUMBIA UNIVERSITY,
April, 1907.

A PECULIAR LEGLESS SHEEP.

CHARLES R. STOCKARD.

A peculiar sport lately appeared in a flock of sheep in Nash County, N. C.[1] During the early part of February a black ewe gave birth to a lamb by a white ram; the offspring was entirely devoid of all external indications of limbs and was black in color like its mother. This lamb has a perfect head and body with the usual long tail, and has been in a healthy condition since birth. It was fed on milk from a bottle for the first month or two, but is now able to eat grass and appears normal in size and other respects except for its apodal condition. The movements of this animal are limited; it can right itself if turned on its back and can twist its body about to some extent, but is entirely incapable of any progressive movements (Figs. 1 and 2, Pl. XIII.).

Photographs and descriptions of the lamb indicate that it is a sport or mutation rather than a merely deformed monster.

Another legless lamb almost identically like the first one was born in this same flock three months later. The two lambs had the same father (there being only one ram with the flock), but different mothers. This second lamb was a white male, and unfortunately was killed.

The parent ram is an old sheep, but no definite or authentic records of his previous offsprings can be obtained. It is a peculiar coincidence, however, that two of his young in so short a time should have shown this remarkable legless condition. Such a fact suggests, from the rather insufficient evidence, that this male has within his germ-cells a tendency to produce these lambs without legs.

The occurrence of the legless animals recalls the classical case of the ancon ram, a sheep with short crooked legs, that was born in Massachusetts about 1791. The ancon race was produced from this one ram by crossing at first with common sheep. The

[1] I have not up to this time been so fortunate as to personally observe these sheep, but the photographs and descriptions have been obtained from an entirely reliable source.

legless sheep here recorded seem to have carried such a variation a last step further, and it will be extremely interesting to know whether this character will tend to establish itself and produce a legless race.

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Explanation of Plate XIII.

Fig. 1. Shows a photograph of the apodal lamb with its ventral surface turned upward; the animal lying on its back.

Fig. 2. The lamb with its ventral surface toward the reader. Both photographs serve to show the entire absence of external limbs.

FIG. 1.

FIG. 2.